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ANNALS OF BOTANY

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ERRATA

Vol. XLIII, no. CLXXII, p. 810, Table IV :

for $S(x-\bar{x})$ read $S(x-\bar{x})^2$

for $n^2 + 1$ read $n_1 + 1$.

Vol. XLIV, no. CLXXIV, p. 459, lines 4, 5, and 6 :

Insert \pm *between* grm. and 0.010

grm. and 0.011

grm. and 0.009.

P. 461, Table III, column 6, *omit* a in the first three lines.

P. 462, Table IV, *for* 0.0155 $e^{0.9965t}$. *read* 0.0155 $e^{0.0965t}$.

Studies on the Transport of Nitrogenous Substances in the Cotton Plant.¹

III. The Relation between Longitudinal Movement and Concentration Gradients in the Bark.

BY

E. J. MASKELL

AND

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With five Figures in the Text.

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SECTION I. INTRODUCTION.

IN Part I (1) of this series of papers an account was given of the variation in total nitrogen content of leaf, bark, and wood, in relation to the normal alternation of day and night, and also in response to various forms of ringing. The gross phenomena observed, namely, the spread of diurnal

¹ Paper no. 5 from the Physiological Department of the Cotton Research Station, Trinidad.

changes from leaf to bark, the accumulation of nitrogen in leaves and wood as well as in the bark above a ring, and the type of response shown to partial ringing, bore a close resemblance to the gross phenomena of carbohydrate transport. The results suggest that in both cases transport is determined by concentration gradients. For carbohydrate transport this suggestion had been already confirmed by the results of a study of sugar concentrations in the sap (3, 4). The normal downward movement is along a sugar concentration gradient in the bark, and the rate of movement is approximately proportional to the gradient.

When we came, however, to study the vertical gradients in nitrogen compounds associated with the normal downward transport of nitrogen, we found (2) that downward movement takes place in the presence of marked negative gradients of total N and total organic crystalloid N. There appeared to be at least two possible explanations, either that some only of the crystalloid N fractions were concerned in transport, and that their gradients were positive but were masked by negative gradients of the storage fractions (chemical masking), or else that positive gradients of mobile compounds in the actual conducting channels were masked by negative gradients of the same compounds in the storage tissues (regional masking). Chemical analysis showed that asparagine and amino-acids had marked negative gradients, and that the gradient in these two fractions combined was, in general, more than sufficient to account for the total negative gradient in organic crystalloid N. This suggested that some fraction included in residual N might have a positive gradient; but, although small positive gradients in residual N were indicated, these were in no case statistically significant.

The hypothesis that there is a positive gradient in the sieve-tubes, masked by a negative gradient in the storage tissues (rays and cortex) was tested by studying the vertical gradients in inner and outer halves of the bark. If this simple hypothesis is correct the outer half should show a strong negative gradient and the inner half a smaller negative gradient, or even a positive gradient. For asparagine and amino-acids, however, the inner half showed negative gradients even greater than those in the outer half, while the small positive gradient of residual N was about the same in both halves, and was not significant in either. This simple test of the masking hypothesis is, of course, only valid if ray tissues and cortex have roughly the same negative gradient. If the negative gradient is much steeper in the rays than in the cortex, then the gradient in the sieve-tubes of the inner half will still be strongly masked, and the gradient observed in the outer half of the bark will be no guide to the masking gradient. The possibility of positive gradients in the sieve-tubes still remains open, for even if these gradients are not masked by gradients in the rays, they may still be masked by gradients in the companion cells.

Some evidence as to the concentration levels of different compounds in the different tissues of the bark was obtained by subdividing the bark of one region of stem into three zones—outer, middle, and inner. The data obtained indicated that amino-acids and residual N, like sucrose, have high concentrations in the sieve-tubes and low concentrations in rays and cortex: asparagine, on the other hand, appears to exist in highest concentration in the rays, the concentration in the sieve-tubes, though much higher than in the cortex, being below that in the rays. This spatial distribution of the crystalloid fractions suggested that asparagine may be concerned mainly with storage, while amino-acids and residual N are concerned with transport. Evidence of positive gradients of either of the latter fractions in the sieve-tubes is, however, lacking. The hypothesis that residual N may be concerned in transport receives some support from the fact that there is a steep gradient of residual N out of the leaf-parenchyma into midrib and petiole. The parallel gradient of amino-acids is much smaller, while the small asparagine gradient is in the opposite direction. It seemed just possible, therefore, that residual N might be the head for transport throughout the plant.

Thus, although the data obtained open up a number of interesting possibilities, chemical and regional analyses of the gradients associated with normal downward movement have so far failed to reveal any definite gradient in the direction of movement.

In the present paper we approach the problem from a slightly different standpoint—that of the relation between *change in movement and change in gradient*. We have suggested above that there may be gradients of storage material which mask the gradients of translocatory material. If we stop transport or reverse its direction we should affect only, or mainly, the dynamic gradient of translocatory material. While, therefore, the net gradient may be negative, the *change* in the net gradient, when the rate or direction of transport is altered, should be positively correlated with that change in movement, and should be a measure of the change in the dynamic gradient. Where movement is approximately proportional to the net gradient, as appears to be the case for sugars, there can be no appreciable storage or static gradient; and the net gradient when movement stops should become zero. Where there exists also a static gradient, only the dynamic gradient will disappear at zero movement, and the net gradient will then be equal to the static gradient. We attempt, therefore, to separate the static and dynamic components by studying the change in gradients (*a*) when movement is brought to standstill, (*b*) when the direction of movement is reversed.

SECTION 2. GRADIENT CONDITIONS ASSOCIATED WITH CESSATION OF TRANSPORT (EXPERIMENT 7).

The object of this experiment was to ascertain the changes in the vertical gradients in the bark which occur when the transport of nitrogen is brought to a standstill by ringing the stem at its base and removing the leaves. First, the gradients in the normal or untreated plants were determined. The approach to static equilibrium conditions in the leafless plants was then followed by determining the gradients after five and eight days respectively. A group of plants (Leaves-on group) ringed at the base of the main-axis, but bearing leaves, acted as a control on the behaviour of the leafless group (Leaves-off group).

(a) Procedure.

The plants were about 100 cm. high and were without flowers or bolls. The main-axis was marked with wool so as to divide it into four regions, as shown in the diagram (Fig. 1). The *Lower region* of the stem extended from one inch above the ground level to the first fruiting-branch; next there was an intermediate region of seven internodes and above this an *Upper region* of six internodes. The whole apical region above was removed. Leaves and branches were removed from the Lower and intermediate regions before the experiment began. On the first day of the experiment both groups of plants were ringed one inch above the ground and the leaves were removed from the Upper region of the Leaves-off group. An initial collection of four samples of normal or untreated plants was also made on the first day. After five days two samples were taken from each group; an additional pair of samples was taken three days later. Each sample consisted of twenty-five plants.

The samples taken for analysis consisted of the bark and wood of the Upper and Lower regions; the sections of stem were trimmed at the stipular marks before weighing. Upper and Lower regions are shaded in the diagram. The time table shows the sequence of operations:

Time Table.

December, 14.	6.15 a.m.	Ringing and removal of Leaves.	
	8.45 a.m., 10.15 a.m. }	4 Collections from Normal Plants.	
	1.0 p.m., 2.20 p.m. }		
19.	9.15 a.m., 1.10 p.m.	2	„ of Leaves-on Plants.
	10.45 a.m., 2.20 p.m.	2	„ „ Leaves-off „
22.	8.30 a.m., 12.50 p.m.	2	„ „ Leaves-on „
	10.05 a.m., 2.15 p.m.	2	„ „ Leaves-off „

Determinations were made of total N, crystalloid N, sucrose and reducing sugars.

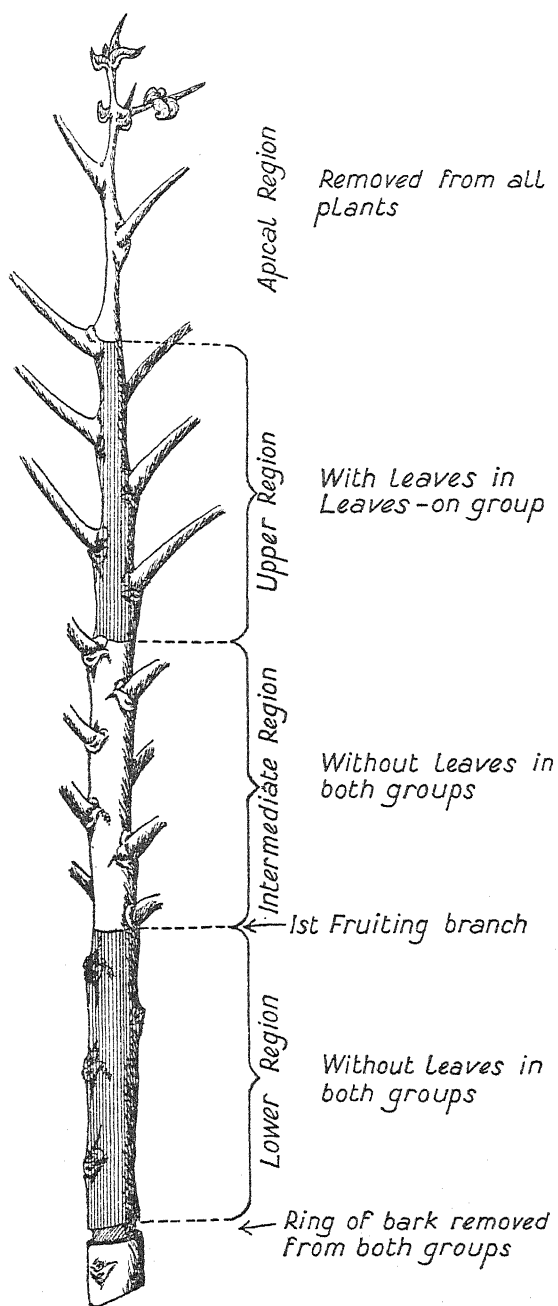


FIG. 1. Illustrating treatment of stems for Experiment 7.

(b) Results.

(1) Changes in dry weight and total nitrogen.

Before considering the changes in concentration and in gradients, it will be well to note the changes in total N content and in dry weight. The

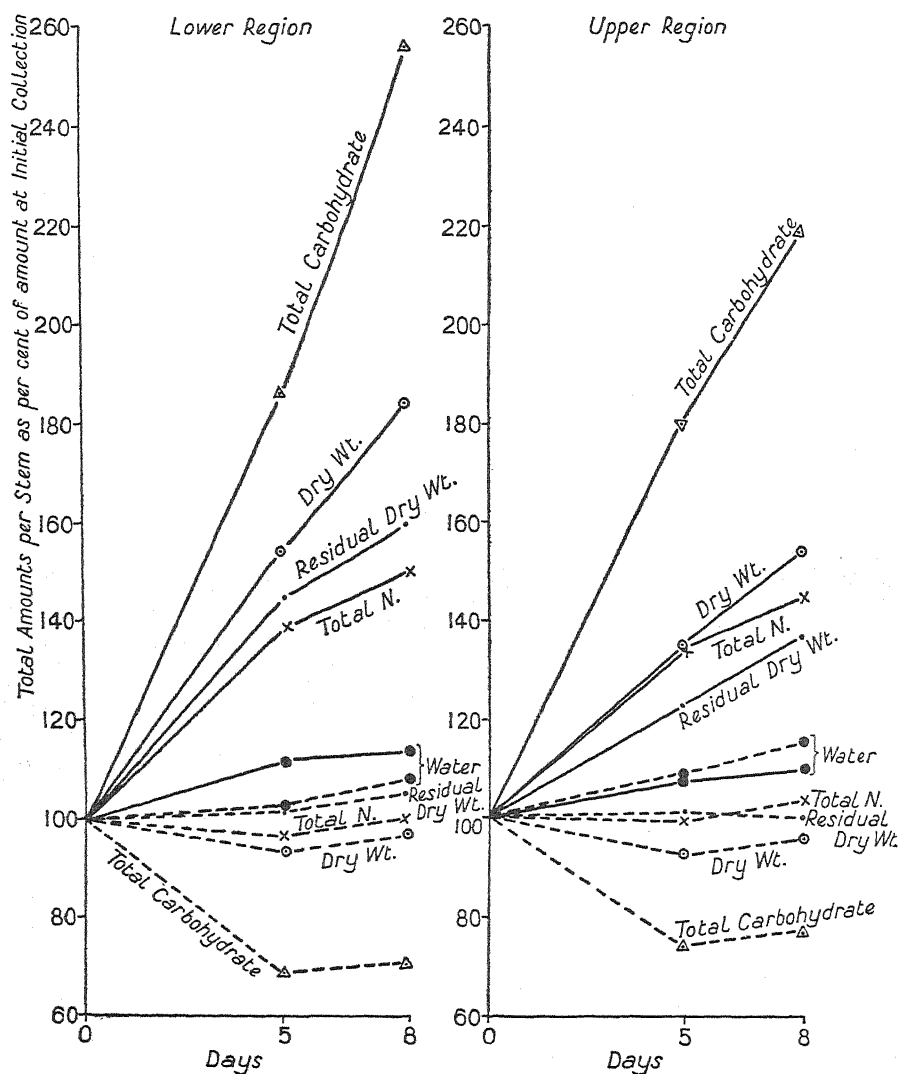


FIG. 2. Changes in dry weight, residual dry weight, total N, total carbohydrate and water. Leaves-on group continuous line. Leaves-off group broken line.

values of total N, total carbohydrates, water, dry weight and residual dry weight per stem (bark and wood) at each collection are shown graphically in Fig. 2. The values are plotted as a per cent. of the corresponding value

for the initial collection. It will be seen that a considerable amount of growth has taken place in the Leaves-on group. There is a large accumulation of carbohydrates (total sugars and polysaccharides), and the residual dry weight also has increased. The nitrogen content has increased considerably, but the relative increase is less than the relative increase in dry weight, the divergence being most marked in the Upper region. In comparison the increase in total water per sample is relatively very small.

In the Leaves-off group total N and the residual dry weight show very little change. Total carbohydrates, however, have decreased considerably so that the total dry weight has also diminished. Water content, on the other hand, has definitely increased,¹ the increase in the Upper region being actually greater than the increase in the same region of the Leaves-on group. The increase in dry weight and in total N in the Lower region of the Leaves-on group indicates that there was in these plants a downward movement of carbohydrates and also of nitrogen, for this region bore no leaves. In the Leaves-off group, on the other hand, there was no appreciable downward movement of nitrogen.

(2) *Changes in concentrations and gradients.*

The concentrations in the Upper and Lower regions of the bark are shown in Fig. 3. The changes in sugar concentration are what would be anticipated on the basis of previous work. In the Leaves-on group the concentration increases markedly in the Lower region (i. e. just above the ring) and approaches the concentration in the Upper region, which also rises, although to a much smaller extent. Synthesis of sugar in the leaves attached to the Upper region is, however, still proceeding, and the positive gradient is maintained to the end of the experiment. The maintenance of the positive gradient is particularly clear for sucrose. The Leaves-off group shows a fall of total sugar concentration in both regions. In the Upper region, which had initially the higher concentration, the fall is the more rapid, and it continues until the concentration here reaches the same low level as in the Lower region. Sucrose shows a parallel behaviour. The results indicate very strongly that at static equilibrium (i. e. when transport stops), the concentration of sugar is approximately the same at different vertical levels in the bark.

There is a marked contrast when we turn to nitrogen. The vertical gradients show very little change, and in the Leaves-off group at the end of the experiment we are still left with a definite negative gradient, both in total crystalloid N and in protein N.

The gradients, with their standard deviations, are given in Table I. The mean gradient in total N in the Leaves-off group is not significantly

¹ It is of interest to note that the bark lost water in the Leaves-off group while the wood gained, the gain by the wood outweighing the loss from the bark.

different from the initial negative gradient. The negative gradient in the Leaves-on group, however, has definitely diminished. The changes in the protein gradient are smaller and are not significant. The negative gradient in total crystalloid N diminishes both in the Leaves-on and in the Leaves-off

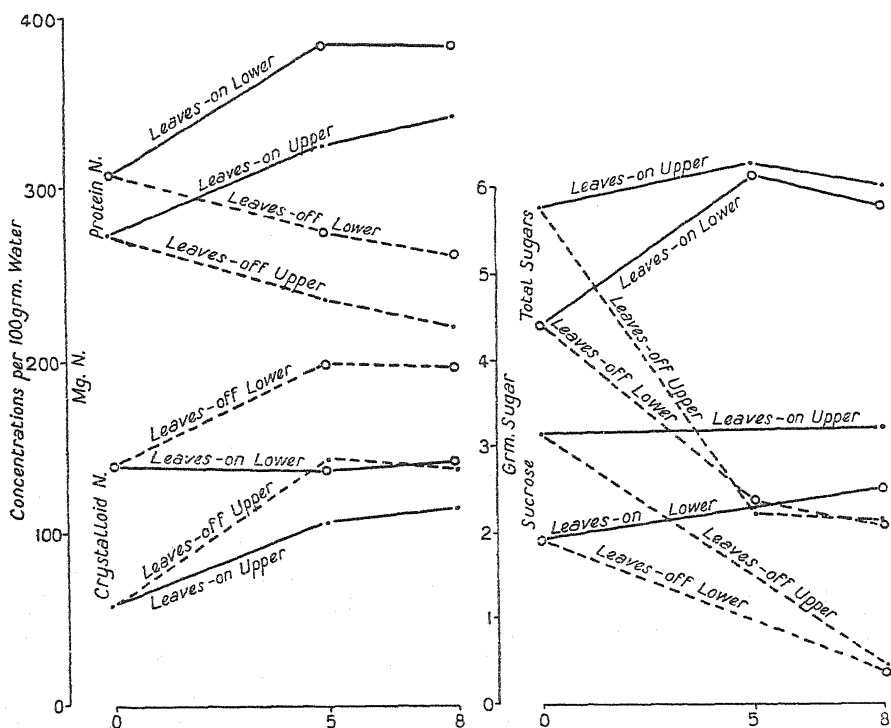


FIG. 3. Changes in concentration of nitrogen compounds and of sugars.
Leaves-on group continuous line. Leaves-off group broken line.

groups ; but, whereas the change in the Leaves-on group is considerable and fully significant, that in the Leaves-off group is barely significant. The difference between the two groups is, however, quite significant. Complete interpretation of the changes observed is not as yet possible, but the results may be regarded as establishing the following facts: (1) When, as in the Leaves-off group, movement of nitrogen is brought to a standstill, there still remains a marked negative gradient in crystalloid N and in protein N concentration ; (2) where downward movement of nitrogen is still proceeding, as in the Leaves-on group, the negative gradient in total N and in total crystalloid N is smaller than where downward movement is at a standstill, as in the Leaves-off group. In other words, the change from zero movement to normal downward movement is associated with a positive change in the gradient, the mean change being +19.8 mg. per cent. for total N and +30.0 mg. per cent. for total crystalloid N.

TABLE I.
Vertical Gradients in the Bark.

(mg. per 100 grm. water).

	Total N.	Protein N.	Crystalloid N.
Initial Collection . . .	-114.1	-33.2	-80.9
Leaves-on 1st Collection . . .	-89.0	-58.9	-30.1
„ 2nd „ . . .	-65.0	-40.7	-24.3
„ Mean „ . . .	-77.0	-49.8	-27.2
Leaves-off 1st Collection . . .	-93.5	-37.9	-55.6
„ 2nd „ . . .	-100.0	-41.2	-58.8
„ Mean „ . . .	-96.8	-39.6	-57.2
Standard Deviation of Mean . . .	10.8	9.33	7.5
Significant Difference! $P = 10.05$.	36.3	31.4	25.2
between Means $P = 10.10$.	29.8	25.7	20.7

The observed gradient in the bark may thus be regarded as consisting of a dynamic gradient of translocatory nitrogen superimposed on a static gradient of storage nitrogen, the dynamic gradient being positive in the Leaves-on group, where downward movement is proceeding, but approximating to zero in the Leaves-off group, where downward movement has stopped. The gradient in the Leaves-off group would then be a *measure* of the static gradient due to storage nitrogen, and the difference between the Leaves-off and the Leaves-on groups would be a measure of the dynamic gradient in translocatory nitrogen.

A difficulty in the way of this interpretation is that the negative gradient in the Leaves-off group is smaller than the negative gradient at the initial collection. It is true that the difference is hardly significant and might be due to sampling: it is also true that we have no evidence as to rate or direction of movement at the time of the initial collection. It seems, however, reasonable to assume that downward movement was then proceeding; But the rate must have been small, as there was no appreciable gain by the Lower region of the Leaves-off group at the expense of the Upper region (Fig. 2). Now if, as in comparing the final gradients in the Leaves-off and Leaves-on groups, we assume that the gradient in the Leaves-off group is a measure of the static gradient, we should calculate for the initial collection a dynamic gradient in total crystalloid N of -23.7 mg. per cent., or for total N -17.3 mg. per cent., i. e. a negative gradient in translocatory nitrogen. It is of course very probable that the conditions which determine the vertical distribution of storage nitrogen, and hence the static gradient, may have altered in the five to eight days since the initial collection. But this will not resolve the difficulty, for the very different conditions obtaining in Leaves-on and Leaves-off groups may equally have produced a difference between the static gradients in these two groups. The negative gradient in the Leaves-off group would then be no reliable measure of the static (storage) component of the observed gradient in the Leaves-on group.

The data are insufficient to establish any complete interpretation. The broad facts however remain that, when movement of nitrogen is brought to a standstill, there is still a negative gradient in the bark; and that this negative gradient is definitely greater than the negative gradient where downward movement is still proceeding. In the case of carbohydrate transport, on the other hand, as downward movement comes to a standstill the vertical gradient falls to zero. The formal interpretation of these results in terms of a static gradient, determined by regional differences in storage conditions, and a dynamic gradient of translocatory nitrogen must await further experimental testing.

It will be noted that the crystalloid fractions were not determined in this experiment. This limitation of the procedure was due to the feeling that it might be better first of all to determine the characteristic behaviour of the two primary nitrogen fractions, i. e. protein and crystalloid N, before proceeding to study the characteristic behaviour of the individual crystalloid fractions. It seemed that progress might be more certain if some definite decision as to the general role of the crystalloid as against the colloid or protein N compounds could be arrived at. For the same reason, in certain ringing experiments to be described in a later paper, only protein N and total crystalloid N were determined. But even this apparently simple question is very difficult to answer. The difficulty arises in part from the very great fluctuations which take place in the relative proportions of protein and crystalloid N. Thus, in this experiment, although the *total* N content of the bark of the Leaves-off group remains practically constant yet the crystalloid N increases very considerably, while protein N shows a corresponding decrease. At the initial collection the total crystalloid N forms on the average 25 per cent. of the total N. In the Leaves-off group it increases to 40 per cent. of the total N. This increase is clearly due to the conversion of protein into crystalloid N. The increase in crystalloid N in the Upper region of the Leaves-on group (Fig. 3), on the other hand, is probably due to fresh increments of organic N from the leaves. This increase is, however, relatively small, the greater part of the additional nitrogen appearing as protein. The concentration of crystalloid N is in consequence very much greater in the Leaves-off than in the Leaves-on group.

With regard to the conditions determining the gross ratio of crystalloid to protein N, it may be pointed out that the increase in the proportion of crystalloid N in the Leaves-off group is associated with a marked decrease in the concentration of sugars. The association of carbohydrate supply with the synthesis and breakdown of protein is fairly well known (5). The question of the lability of the crystalloid-protein ratio and the bearing of this lability on transport will be discussed more fully in a subsequent paper.

SECTION 3. GRADIENT CONDITIONS ASSOCIATED WITH REVERSAL OF THE NORMAL DIRECTION OF TRANSPORT (EXPERIMENT 9).

The results just considered support the view that there is a static or storage component in the observed vertical gradients of nitrogen in the bark: for at zero movement there is still a marked negative gradient of total N and total crystalloid N. In the experiment described below we make a further test of this conception by studying the gradients in the stem of two groups of plants, when the direction of movement in one group is upward and in the other downward. We have already suggested that the static component may be due to a relatively permanent gradient in the storage tissues of the bark; it is also possible that certain nitrogen compounds may be mainly concerned in storage, while others are of greater importance for transport. Consequently in the present experiment we have endeavoured to obtain information on the gradients in different zones of the bark and also on the chemical composition of these gradients. As this involved a large increase in the number of plants handled and in labour, in neither direction could the analysis be pushed as far as we should have wished. The bark was subdivided into two zones, inner and outer, and the total crystalloid N was separated into asparagine N, amino-acid N, ammonia N, and a residual N + nitrate N fraction. Nitrate determinations could not be carried out owing to a shortage of sap.

The arrangement of the experiment differs in certain important respects from the Reversal experiment described in our work on carbohydrate transport (4). Instead of arranging, as in that experiment, that one specified region of stem should receive food material in one case from above and in the other case from below, two contiguous regions of stem, an Upper and a Lower, were used. In one group of plants the Upper region is made to supply nitrogen and carbohydrates to the Lower; in the other group the Lower region supplies to the Upper. This enables us to eliminate any permanent static gradient between Upper and Lower regions, for the Upper and Lower regions are equally receiving and supplying regions.

(a) Procedure.

Some days before the experiment began the plants were marked with wool so as to divide the main-axis into four regions as shown in the diagram (Fig. 4). The apical region extended from the apical bud downwards to the 11th node. Below this were the *Upper* region, which included five nodes, and the *Lower* with seven. The stem below the Lower region was bared of leaves and branches. There were three groups of plants. On the day the experiment began a quarter-inch ring of bark was removed immediately above the Upper and immediately below the Lower regions in all three groups (levels C and A, Fig. 4). The Upper and Lower regions of stem

were thus isolated from the rest of the plant. In the *Leaves-high* group the leaves were removed from the main-axis and branches of the *Lower* region. In the *Leaves-low* group they were similarly removed from the *Upper* region. In the Ringed group all leaves were removed from both regions, and an additional ring of bark was removed between the Upper and Lower regions (level B, Fig. 4); Upper and Lower regions were thus isolated from one another in this group. The reasons for these operations are probably evident. In the Leaves-high group the Upper region of stem, bearing leaves, should supply sugars and organic nitrogen to the Lower region, while in the Leaves-low group the Lower region should supply the Upper. The rings above the Upper region and below the Lower region were, of course, intended to prevent movement of organic food material to the apical region or to the roots. The Lower region of stem in the Ringed group serves as a base line for the measurement of the downward movement into the Lower region of the Leaves-high group. The Upper region of stem in the Ringed group serves similarly as the base line for the measurement of any upward movement into the Upper region of the Leaves-low group.

In sampling the stems the plants were cut in the field at the levels A, B, and C (Fig. 4), and the Upper and Lower regions of stem brought into the laboratory. The stem samples were then trimmed at the stipular mark of the nodes next to the cut ends so that the Upper region consisted of four internodes, and the Lower of six.¹ The bark of the trimmed samples was then subdivided into inner and outer zones, the fresh weight of each zone and of the wood determined, and samples taken for freezing and for moisture determinations.

The determinations made were: sucrose and reducing sugars, total N, total crystalloid N, asparagine N, amino-acid N, and ammonia N. Nitrate N was not determined owing to insufficiency of sap.²

In each sample taken there were fifty-four plants, and two such samples were taken at each collection. The time table below shows the sequence of operations:

Time Table.

January 16, 1928,	6.30–7.30 a.m.	Ring of Plants at levels A and C.
	7.30–8.30 a.m.	Removal of Leaves from Upper and Lower regions and ringing at level B in Ringed group.
	9.0 a.m.	Initial Collection of Normal Plants (2 Samples).
January 18, 1928.		Collection of Leaves-high, Leaves-low, and Ringed groups.
	9.20 a.m.	Collection of 1st Samples.
	1.22 p.m.	„ „ 2nd „

¹ The portions of the stem removed in trimming were preserved in alcohol and used for the microscopical examination of the composition and area of the bark tissues.

² Details of the methods of estimation used have been given in Part II (2) q.v. The determinations of total N and of crystalloid N include nitrate N. Asparagine N is taken as twice the amide N. Amino-acid N is the total amino N (Van Slyke), less an amount equal to the amide N of asparagine. Owing to the fact that there may be amides other than acid amides in the sap the figure for amino-acid N is a minimal one.

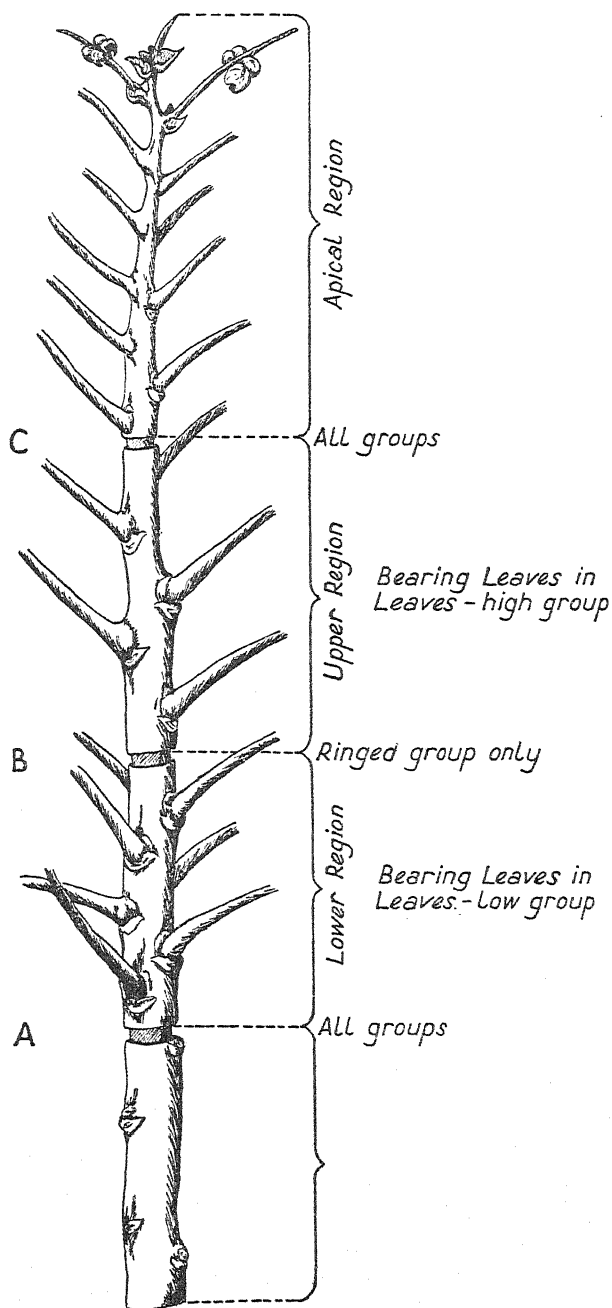


FIG. 4. Illustrating treatment of plants for Experiment 9.

(b) *Results.*(1) *Changes in dry weight and total nitrogen.*

A summary of the results for the Upper and Lower regions of each group of plants is given in Table II. The values given are the means for the two samples collected, and represent the weight per plant, the amount actually determined per sample being divided by the number of plants in the sample.

Since there was no significant difference between the relative variances for Upper and Lower regions these variances have been combined in order to have a general estimate of sampling variation. This estimate is given at the foot of the table in the form of the standard deviation, expressed as a percentage of the mean for any pair of duplicate samples.

TABLE II.

Grm. Carbohydrate and mg. N per plant in Upper and Lower regions of Stem (Bark and Wood).

	Dry Weight.	Total N.	Total. Carbohydrate.	Residual Dry Weight
<i>Upper Region:</i>				
Initial	9.337	84.91	2.511	6.826
Ringed	9.398	89.80	2.228	7.170
Leaves-high . . .	10.572	105.50	3.115	7.457
Leaves-low . . .	10.322	107.43	2.898	7.424
<i>Lower Region:</i>				
Initial	20.428	174.69	5.030	15.398
Ringed	20.530	187.54	4.944	15.586
Leaves-high . . .	22.393	220.20	6.126	16.267
Leaves-low . . .	22.955	227.69	6.302	16.653
Standard deviation (As per cent. of the mean)	1.15	1.93	1.93	1.01

The significance of the changes observed may be judged from Table III, in which the difference between any two groups is expressed as a per cent. of the mean for the two groups. Statistically significant changes are shown by the figures in heavy type.

The most important result is that shown by the two sets of differences—Leaves-low minus Ringed for the Upper region, and Leaves-high minus Ringed for the Lower region. The first measures the upward movement in the Leaves-low group, the second the downward movement in the Leaves-high group. It was shown in an earlier paper (4) that the normal downward movement of carbohydrates in the bark may be reversed by reversing the relative positions of 'source' and 'sink'. It is clear from this experiment that the normal downward movement of nitrogen may similarly be reversed. It will be noticed that not only the dry weight, but also the residual dry

weight of the plants bearing leaves show a significant increase over the Ringed group :

TABLE III.
Relative Differences between Groups.

	Dry Weight.	Total N.	Total Carbohydrate.	Residual Dry Weight.
<i>Upper Region :</i>				
Ringed minus Initial.	+0.65	+5.60	-11.95	+4.92
Leaves-high minus Leaves-low. .	+2.39	-1.81	+7.20	+0.44
Leaves-low minus Ringed	+9.37	+17.88	+26.16	+3.48
<i>Lower Region :</i>				
Ringed minus Initial	+0.50	+7.10	-1.71	+1.21
Leaves-low minus Leaves-high. .	+2.48	+3.34	+2.83	+2.35
Leaves-high minus Ringed	+8.68	+16.02	+21.34	+4.28
Significant Difference $P = 10.05$	3.846	6.294	6.294	3.382
" " $P = 10.10$	3.082	5.077	5.077	2.710

A result of secondary interest is the set of differences shown in the Upper and Lower regions between the Leaves-high and the Leaves-low groups. The Upper region in the Leaves-high group is *supplying* nitrogen and carbohydrates to the Lower region ; the corresponding region in the Leaves-low group is *receiving* nitrogen and carbohydrates from the Lower region. Consequently, assuming supply from the leaves to be approximately the same in the two groups, we should expect the Upper region of the Leaves-high group to exceed the Upper region of the Leaves-low group in carbohydrates (or dry weight) and in total N. In the Lower region the Leaves-low group should exceed the Leaves-high group. It will be seen that in the Lower region all the differences observed are in the expected direction. None of these are, however, statistically significant. In the Upper region dry weight and total carbohydrates show the expected differences, the carbohydrate effect being quite significant. The difference in total N is in the wrong direction, but is insignificant. It will be evident that on the whole the facts fit into a gradient picture of reversed movement.

The third set of comparisons, that between the Ringed and the Initial groups, presents some puzzling features. It is not essential for the measurement of movement in the bark, for this depends only on the comparison between the samples of the three groups collected at the end of the experiment. The results differ, however, in certain respects from those of previous experiments, and some comment is desirable. It will be seen that, although the ringed stem shows a decrease on the initial collection in total carbohydrates, the dry weight shows a slight increase,¹ while total N shows a quite appreciable increase. This increase in nitrogen is quite significant in the Lower region, and has a significance between $P = 0.10$ and $P = 0.05$ in the Upper region. In the absence of fresh supplies of carbohydrates one would

¹ The increase in dry weight is due entirely to the wood, for the bark shows a definite loss.

have expected a net loss of dry weight owing to loss in respiration. Thus, although the net increase in dry weight is not statistically significant, the fact that there is an increase, rather than a decrease, suggests that carbohydrates must have entered this region of stem, *via* the wood. The amount of movement involved is, as will be seen from Table II, very small compared with the movement that occurred in the bark of the unringed groups, and the data would be consistent statistically with an entire absence of any such movement. If the suggested movement of carbohydrates did take place it seems probable that it was due to small quantities of sugar brought up in the transpiration current.

Similarly the undoubted increase in nitrogen content probably represents a gain of nitrates from the upward moving transpiration stream. Whether there was any appreciable conversion of this nitrate to organic nitrogen in the bark or wood cannot be decided, since no nitrate determinations were carried out. It should be said, perhaps, that this is the only case so far observed in our work in which a gain in nitrogen or in dry weight is indicated for a defoliated region of stem between two rings. In all other cases dry weight has shown a loss and nitrogen either no change or a very small loss.

Returning now to the main question, the reversal of the direction of movement in the bark, it will be of interest to consider how much movement of carbohydrates and of nitrogen has taken place. As a measure of the amount of carbohydrate transported we take the increment of dry weight less 5.7 times the increment of nitrogen. This allows for the increase in dry weight due to the parallel transport of nitrogen compounds, which we assume to have about the same proportion of nitrogen to dry weight as asparagine. The actual amounts transported per plant are then as follows:—*Downward transport*: Total N, 32.67 mg.; dry weight, 1.863 grm.; dry weight less 5.7 N, 1.678 grm. *Upward transport*: Total N, 17.63 mg.; dry weight, 0.924 grm.; dry weight less 5.7 N, 0.822 grm.

(2) *Changes in concentrations and gradients.*

The accompanying changes in concentrations and in vertical gradients in the bark may now be considered. The concentrations for the inner and outer halves¹ of bark are shown in Table IV and the vertical gradients in Table V.

It will be seen that the differences in sugar concentration are mostly due to changes in sucrose, and are most pronounced in the inner half of the bark. Reducing sugars show very little change in either half. In the Upper region of the stem the Leaves-low group shows a decrease in sucrose on the initial collection, and the Leaves-high group an increase. In the lower region of the stem, on the other hand, both groups show an increase in sucrose,

¹ Calculation of concentrations in inner and outer halves from the data for the inner and outer fractions, which do not exactly divide the bark into two halves, is made as explained in Part II (2).

but the increase is somewhat greater in the Leaves-low group. These changes, which occur mainly in the inner zone of the bark, are what would be expected in view of the fact that in the Upper region the Leaves-high group is supplying and the Leaves-low group receiving carbohydrates, whereas in the Lower region conditions are reversed.

TABLE IV.

Concentrations in the Bark.

(Grm. sugar and mg. N per 100 grm. water.)

	Inner Half of Bark.			Outer Half of Bark.		
<i>Upper Region:</i>	Initial.	Leaves High.	Leaves Low.	Initial.	Leaves High.	Leaves Low.
Total N . . .	479.9	580.8	583.7	416.7	499.3	507.7
Protein N . . .	333.1	379.3	364.2	323.8	372.3	378.1
Total Cryst. N . .	146.8	201.5	219.5	92.9	127.0	129.6
Asparagine N . . .	74.2	110.9	123.6	50.1	71.9	74.8
Amino-acid N . . .	22.6	31.9	19.7	13.6	15.7	17.2
Residual N + } Nitrate N	49.8	55.7	73.9	27.6	36.9	33.4
Ammonia N . . .	0.2	3.0	2.3	1.6	2.5	4.2
Total Sugars . . .	6.575	7.024	6.144	6.259	6.345	5.707
Sucrose	4.149	4.473	3.656	2.437	2.474	1.992
Reducing Sugar . .	2.426	2.551	2.488	3.822	3.871	3.715
<i>Lower Region:</i>						
Total N	504.6	619.5	656.4	463.0	541.7	556.5
Protein N	303.4	387.9	400.4	345.7	392.0	397.5
Total Cryst. N . . .	201.2	231.6	256.0	117.3	149.7	159.0
Asparagine N	123.9	142.6	160.0	76.1	96.9	105.4
Amino-acid	30.0	33.0	39.3	17.0	17.1	11.8
Residual N + } Nitrate N	45.5	52.8	53.8	22.7	30.6	36.9
Ammonia N	1.8	3.2	2.9	1.5	5.1	4.9
Total Sugars	6.002	6.425	6.502	5.698	5.256	5.449
Sucrose	3.428	3.886	3.924	2.134	2.013	2.070
Reducing Sugars . .	2.574	2.539	2.578	3.564	3.243	3.379

The changes in the vertical gradients (Table V) are even more striking. Taking first the inner half of the bark, we note that the normal positive gradient of total sugars increases somewhat in the Leaves-high group, with its positive or downward movement of carbohydrates, whereas in the Leaves-low group, where the direction of movement is negative (i. e. upward), the gradient also is negative. In the outer half of the bark, on the other hand, the vertical gradient of total sugars in the Leaves-low group is still *positive*, although it has diminished since the initial collection, and is very much smaller than the positive gradient in the corresponding zone of the Leaves-high group. Reversal of the direction of movement of carbohydrates is thus associated with reversal of the concentration gradient in the inner half of the bark, but not in the outer half. The gradient in the bark as a whole is -0.042 per cent. sugar, i. e. just negative but so small that, from this

figure alone, reversal of the gradient could hardly be argued. In our previous work (4) on the reversal of movement of carbohydrates in the bark, in which only the intact bark was handled, clear evidence was obtained of reversal of movement, but the vertical gradient in the bark, although considerably diminished was not definitely reversed. In discussing that experiment it was suggested that reversal of the gradient had occurred in the sieve-tubes, but that this change was masked by the persistence of the original gradient in the other tissues of the bark. The present results, it is clear, completely confirm that explanation, and strengthen considerably the general case for longitudinal transport of carbohydrates along concentration gradients in the sieve-tubes.

The case of nitrogen, as we have seen, is more complex, for we have not been able to establish the existence, during normal downward movement, of a definite positive gradient in any nitrogen fraction, either in the bark as a whole or in the inner zone. We will first, as in the case of sugars, compare the concentrations in the Leaves-high and Leaves-low groups in the Upper and Lower regions of the stem. In the Upper region there is, for the outer half of the bark, very little difference in total N between the two groups. The Leaves-low group shows a somewhat higher concentration in all fractions except nitrate N + residual N. In the inner half of the bark also there is very little difference in total N concentration between the two groups, but there are appreciable differences in the composition of the total N. Protein and amino-acids are relatively low in the Leaves-low group, but asparagine and the nitrate N + residual N fraction are relatively high. That is to say amino-acids and protein behave like sucrose, in showing a lower concentration in that region which is receiving food material. Asparagine and nitrate N + residual N show the opposite behaviour.

In the Lower region of bark the differences in nitrogen concentration between Leaves-high and the Leaves-low groups are greater than in the Upper region, and the Leaves-low group has a higher concentration in every fraction, with the exception of ammonia (both inner and outer halves) and amino-acids (outer half only). As in the Upper region, the greatest relative difference is in amino-acids, while nitrate N + residual N is only slightly different. This increase in concentration of the nitrogen fractions shown by the Leaves-low group is mostly in the inner half of the bark.

It will be seen that there are certain points of similarity between nitrogen compounds and sugars. In both cases concentrations in a region of stem which is supplying nitrogen and sugars tend to be greater than those in the corresponding region of stem which is receiving nitrogen and sugars, and in both cases this concentration effect is mainly found in the inner half of the bark. There are, of course, many differences. In total sugars the greatest difference in concentration between supplying and receiving regions is in the Upper region, in total N the greatest difference is in the Lower

region. Again, the results for the different nitrogen fractions are somewhat confusing, as compared with the consistent behaviour of sucrose. Protein and amino-acids behave like sucrose in both regions; asparagine and nitrate N + residual N behave differently in Upper and Lower regions.

TABLE V.

Vertical Gradients in the Bark.

(Grm. sugars and mg. N per 100 grm. water.)

	Inner Half of Bark.			Outer Half of Bark.		
	Initial.	Leaves High.	Leaves Low.	Initial.	Leaves High.	Leaves Low.
Total N . . .	-24.7	-38.7	-72.7	-46.3	-42.4	-48.8
Protein N . . .	+29.7	-8.6	-36.2	-21.9	-19.7	-19.4
Total Cryst. N . .	-54.4	-30.1	-36.5	-24.4	-22.7	-29.4
Asparagine N . . .	-49.7	-31.7	-36.4	-26.0	-25.0	-30.6
Amino-acid N . . .	-7.4	-1.1	-19.6	-3.4	-1.4	+5.4
Residual N + } Nitrate N }	+4.3	+2.9	+20.1	+4.9	+6.3	-3.5
Ammonia N . . .	-1.6	-0.2	-0.6	+0.1	-2.6	-0.7
Total Sugars . . .	+0.573	+0.599	-0.358	+0.561	+1.089	+0.258
Sucrose . . .	+0.721	+0.587	-0.268	+0.303	+0.461	-0.078
Reducing Sugars .	-0.148	+0.012	-0.090	+0.258	+0.628	+0.336

Coming now to the effect of these concentration changes on the vertical gradients, it will be remembered that, in the case of sugars, the effect in the Leaves-low group was to turn an initial positive gradient into a negative gradient. In the case of nitrogen compounds the initial gradient is already, in most of the fractions, negative, so that reversal of the gradient is not to be expected. It will be seen, however, that the initial negative gradient becomes much steeper in the Leaves-low group, i.e. the *change* in gradient associated with the reversal of movement is in the same direction as is the case for sugars. In terms of the analysis, previously suggested, into static and dynamic gradients, this change would be interpreted as the reversal of an originally positive dynamic gradient of translocatory nitrogen, the static gradient of storage nitrogen being unchanged. It will be seen from the table that all the nitrogen fractions in the inner half of the bark, except the nitrate N + residual N, show this change, the effect being most marked with protein and amino-acids. In the outer half of the bark there are only small changes in gradient.

(3) *The gradient-movement relation.*

The relation between change in gradient and change in movement is shown graphically in Fig. 5. Only gradients in the inner half of the bark are considered. The horizontal axis oo represents zero movement. Positive movement of carbohydrates or of nitrogen (i.e. normal downward movement towards the roots) is measured upwards from this zero line, and negative movement (i.e. reversed movement, upwards in the bark) is measured down-

wards from this zero line. The vertical axis *oo* represents zero observed gradient in the inner half of the bark. Observed gradients when positive are measured to the right from this line, and when negative to the left. The scale for carbohydrates, both for concentrations and for amounts moving, is one-fiftieth of that for nitrogen, i.e. 1 gram. carbohydrate corresponds to 20 mg. nitrogen.

The data plotted are the total amounts of carbohydrates or of nitrogen, per plant, that moved upwards or downwards since the initial collection, and the mean vertical gradients found at the final collections. Strictly speaking, the amounts moved should be plotted against the average gradients during the experiment but, since only initial and final gradients are available, it seemed justifiable in this tentative analysis of the phenomena to consider final gradients only. It will be seen that the line connecting the two points for total sugars passes very nearly through the intersection of the two axes. The lines for the nitrogen compounds lie wholly to the left of the vertical zero axis (except that for residual N + nitrate N). The slope of the line for amino-acid N is almost exactly the same as that for total sugars, while the lines for protein N and total N have a smaller slope. The lines for total crystalloid N and for asparagine N, however, exhibit a very steep slope. The slope of the line for residual N + nitrate N is in the opposite direction, i.e. as movement becomes negative the gradient becomes more strongly positive. The interpretation of the results for this fraction is far from clear. The main feature of the results, namely, the parallel behaviour of sugars, total N, protein N and amino-acid N is, however, very suggestive.

The vertical distance between the two points on each curve represents the total amount of sugar or of nitrogen moved (i.e. the sum of the upward and downward movements); the horizontal distance between them represents the difference between the sum of the concentrations in the supplying regions of the bark and the sum of the concentrations in the receiving regions of the bark. Thus for total N the sum of the concentrations in the supplying regions of the two groups is 1237.2 mg. per cent., and in the receiving regions 1203.2 mg. per cent. The gradient in total N associated with the combined upward and downward movement of nitrogen is therefore 34.0 mg. per cent. As this represents the sum of the gradients between supplying and receiving regions, it may be regarded as the total dynamic gradient.

It will be of interest to look more closely at this relation between the total movement and the total dynamic gradient. From Table VI, which exhibits this relation, it will be seen that the movement of 2.5 gram. of carbohydrates is associated with a final gradient of 0.957 per cent. sugar; hence a total movement of 1 gram. would correspond to a final gradient of 0.383 gram. per cent. If nitrogen and carbohydrates are similar in behaviour the observed movement of 50.3 mg. nitrogen should correspond to a gradient

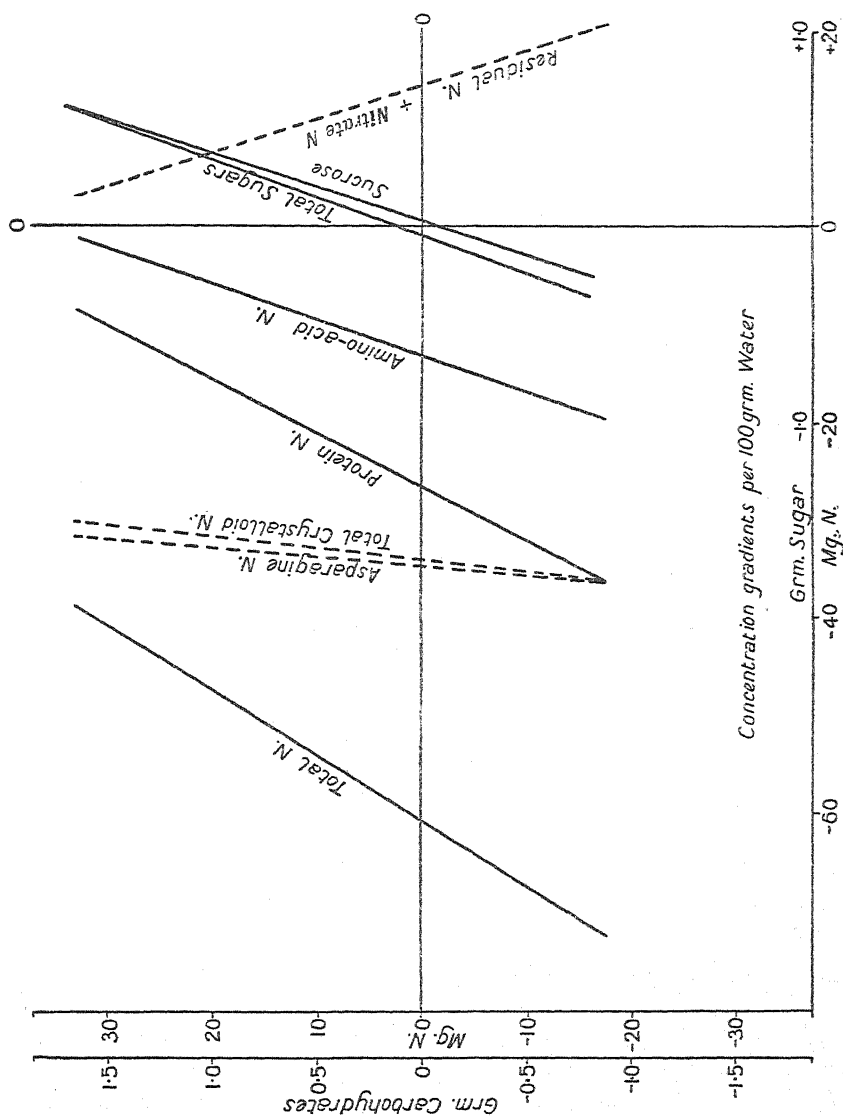


FIG. 5. Relation between gradient and movement. (Experiment 9)

of 50.3×0.383 mg. nitrogen per cent., i.e. 19.3 mg. per cent. It will be seen that the observed final gradients in total N and in protein N are more than adequate for the observed movement, while the gradient in amino-acid N is almost identical with the gradient required. The gradients in the remaining nitrogen fractions would appear quite inadequate. In view of the fact that we have only been able to compare the final gradients, and also that the relation between total gradients in the inner half of the bark and total gradients in the sieve-tube may not be the same for sugars as for nitrogen, any inference drawn must be a tentative one. The results do, however, suggest the possibility that the relation between dynamic gradient and rate of longitudinal movement in the sieve-tubes is approximately the same for sugars as for nitrogen compounds. In that case the mechanism which is responsible for the acceleration of diffusion in the sieve-tubes may act impartially on sugars and nitrogen compounds, and presumably, also on any substance in free solution in the sieve-tube.

TABLE VI.

Total Movement and Total Dynamic Gradient in Inner Half of Bark.

Total Movement of Carbohydrates . . .	2.50 gm.		
Total Dynamic Gradient of Total Sugars . .	0.957 gm. per cent.		
Total Movement of Nitrogen . . .	50.3 mg.		
Expected Total Dynamic Gradient of Nitrogen	19.3 mg. per cent.		
Total Dynamic Gradient of Total N . . .	34.0	"	"
" " " Protein N . . .	27.6	"	"
" " " Total Cryst. N . . .	6.4	"	"
" " " Asparagine N . . .	4.7	"	"
" " " Amino-acid N . . .	18.5	"	"
" " " Residual N + } .	-17.2	"	"
" " " Nitrate N }			

In view of the importance of this tentative conclusion, it will be advisable to make as precise as possible the suggested interpretation of the relation between the observed vertical gradients in the bark and longitudinal movement.

It is assumed that the concentration found in any region of the bark is made up of a part which is relatively immobile, the static concentration, *S*, of storage material, and a part which is freely mobile, the dynamic concentration, *D*, of translocatory material. The total concentration *C* is equal to *S* + *D*. It is further assumed that the static concentration, *S*, has characteristic values at different vertical levels in the bark. These values may alter with age or history of the plant, but two regions at different levels in the bark may always show a characteristic difference in static concentration. In the case of nitrogen, for example, the static concentration would seem to increase as we pass down the stem. The simplest assumption to make in using this conception is that over a short time interval the static concen-

tration is approximately constant and independent of the dynamic concentration. The gradient conditions for the case of the reversal of movement just discussed would then be represented as

	Observed Vertical Gradient.
Leaves-high Group (normal downward movement)	$C_{N.U.} - C_{N.L.} = D_{N.U.} - D_{N.L.} + S_U - S_L$
Leaves-low Group (reversed, i. e. upward, movement)	$C_{R.U.} - C_{R.L.} = D_{R.U.} - D_{R.L.} + S_U - S_L$

where N and R represent the Leaves-high and the Leaves-low groups respectively, and U and L the Upper and Lower regions of the stem. Subtracting the concentrations in the receiving regions $C_{N.L.}$ and $C_{R.U.}$ from those in the supplying regions $C_{N.U.}$ and $C_{R.L.}$, the static concentrations cancel out, and we have the sum of the dynamic gradients associated with the total movement in both directions, $(D_{N.U.} - D_{N.L.}) + (D_{R.L.} - D_{R.U.})$. The relation between movement and dynamic gradient may then be calculated, and from this, knowing the amount of movement in the two directions, the value of $S_U - S_L$ (i. e. the static gradient). This has already been done graphically in Fig. 5, for the slope of the line gives the relation of the dynamic gradient to movement, and the intersection of the line with the zero horizontal axis gives the static gradient. It is important to note that the only assumption necessarily involved is that the static gradient, $S_U - S_L$, is approximately the same in the two groups. The static concentrations themselves need not necessarily be the same in the two groups.

It is probable, however, that the case is not as simple as this. It is more likely that for each region of bark there is a definite relation between the dynamic and the static concentration. The static concentration might stand always in a fixed ratio to the dynamic concentration, or there might be a relationship of the adsorption type. In algebraic terms $S = kD$, k taking characteristic values at different vertical levels in the bark; or else

$S = k_1 \times \frac{D}{k_2 + D}$, k_1 and k_2 or k_1/k_2 taking characteristic values at different vertical levels in the bark. The latter relation seems on the whole the most probable, but the analysis of any experimental results in terms of such an equation would clearly be a matter of some difficulty. It should be noted, however, that solution of the two simpler equations will give an approximation to the limits for the third equation, since when D , the dynamic concentration, is low, S , the static concentration, will be approximately proportional to D , whereas when D is relatively high S will approach saturation value and be almost independent of D .

In order to give objectivity to this form of analysis of concentration gradients we have used the final concentrations observed in the Reversal experiment as estimates of the average concentrations during movement

and have calculated what dynamic and static concentrations would satisfy the equations outlined above. Only the two simple equations (1) $C = D + S$ and (2) $C = D + S = D + kD$ will be used. As examples the concentrations of total sugars, total N and amino-acid N will be considered.

Case 1. $C = D + S$, where S is independent of D and is determined by vertical position only. The following values may be calculated (1) R = grm. substance moved for a dynamic gradient of 1 grm. per cent. (2) $S_U - S_L$ i.e. difference in static concentration between Upper and Lower regions, (3) A minimum value for the static concentration in the Lower region, i.e. assuming that there is zero static concentration in the Upper region. The values obtained are:

	R.	$S_U - S_L$.	(3) Minimum Static Concentration in Lower Region as per cent. of Total Concentration.
Total Sugars	2.613	-0.043 grm. per cent.	0.67 per cent.
Total N	1.478	-60.8 mg. " "	9.55 " "
Amino-acid N	2.719	-13.1 " " "	36.3 " "

Case 2. $C = D + S = D + kD$ or more simply $D = K.C.$, where K is determined by vertical position. In this case we can calculate the ratio of K_U to K_L , these being the values of K for Upper and Lower regions, and also the value of R (i.e. rate of movement for unit gradient) corresponding to any assumed value of K_U (or K_L), neither of which can exceed unity. If we assume that K_U is unity, i.e. that the static concentration in the Upper region is zero we obtain a maximum value for the dynamic gradient and a minimum value for R.

	(1) R Minimum value.	(2) K_U .	(3) K_L .	Static Concentration in Lower Region as per cent. of Total Concentration.
Total Sugar	2.615	1.0	0.993	0.67 per cent.
Total N	1.648	1.0	0.906	9.4 " "
Amino-acid N	3.091	1.0	0.647	35.33 " "

The minimum values obtained for R by equation (2) are very similar to the values calculated by equation (1). It will be seen also from both sets of results that the static concentration in the case of total sugars is negligibly small, and that in the case of total N only a small fraction need be assumed static; a very much larger fraction must be assumed static in the case of amino-acid N.

Using either equation we can give some account of the movement of nitrogen against an apparent gradient in the bark and in both cases, so far as the data go, the acceleration in the rate for unit gradient would seem to be about the same order for nitrogen as for sugars. The method of analysing the data is admittedly formal, and leaves on one side the question of interpreting the static and dynamic components in chemical terms or in terms of localization in different tissues of the bark.

It seems probable, in the present case, that a large part of the static component of the observed gradient is to be interpreted as the gradient in the storage tissues (rays and cortex). For the change in net gradient which takes place when we reverse the direction of movement is mainly localized in the inner half of the bark. We interpret this change in net gradient as being due to the reversal of a dynamic gradient. It follows therefore that the dynamic gradient is present mainly in the inner half of the bark, presumably in the sieve-tubes; while the gradient in the outer half is almost entirely static. We cannot, however, *identify* the static component with the storage gradient in rays and cortex, for there may also be storage or static gradients in the companion cells, and even in the sieve-tubes themselves. But the dynamic vertical gradient is presumably limited to the sieve-tubes.

Parallel in part with this spatial distribution of the total N as between conducting channels and storage tissues, there may also be a chemical distribution as between translocatory compounds and storage compounds. It is possible, on the other hand, that any compound in solution in the sieve-tube may move longitudinally at a rate depending on its gradient, and consequently that longitudinal movement in the sieve-tubes is the resultant of the movement of a number of different compounds, some of which are present also in considerable amount in the storage tissues. The relative importance of different compounds for translocation would then depend only on their relative concentrations in the sieve-tubes, and these might alter with age and history of the plant. As we noted in an earlier paper, amino-acids and residual N appear to be present mainly in the sieve-tubes, while asparagine exists in higher concentration in the rays. We suggested that this might indicate that, while asparagine was concerned mainly in storage, either amino-acids or residual N or both were mainly concerned with longitudinal transport. We drew attention also to a number of other observations which suggested that residual N or some part of it might be the main translocatory form. The behaviour of the nitrate N + residual N fraction in this experiment is, however, quite out of harmony with this view, for the estimated dynamic gradient in this fraction is markedly negative, the concentration being much higher in the receiving than in the supplying regions. It is possible that the anomaly is due to an accumulation of nitrate in the receiving regions, which were, of course, leafless; but it seems improbable that the accumulation of nitrate could have been so great as to mask an opposite variation of the residual N fraction. The facts are strongly against residual N being the translocatory form of nitrogen.

On the other hand, the possibility indicated above, that asparagine is concerned mainly in storage, and amino-acids mainly in transport, receives support from the facts that the dynamic gradient in asparagine is very small, while that in amino-acids is apparently large enough to account for

the nitrogen transported. The greater part of the dynamic gradient in total N, however, is that due to protein N. The presence of sieve-pores suggests that movement of colloid from sieve-tube to sieve-tube is possible, and *a priori* there seems no reason why colloids should not be affected by the same acceleration mechanism as affects crystalloid substances. On this view any substance in free solution in the sieve-tube should move longitudinally at a rate depending on its gradient and its diffusion constant.

One further point must be considered in conclusion. We have seen that, so far as can be judged from the final gradients of total sugars and of total N in the inner half of the bark, the dynamic gradients in total N, nitrogen and amino-acid are adequate for the rate of movement observed if the relation between gradient and movement is the same for nitrogen as for sugars. Since we are using the sugar gradient as a test of the adequacy of the nitrogen gradient, the question arises whether the observed gradient in total sugars in this experiment is of the order to be expected from our previous work (4) on the relation between sugar gradient and movement. If we put $R = kG$ or $G = R \times 1/k$, where R = rate of movement, as gm. per sq. cm. of sieve-tube per hour, and G = gradient of total sugars in the bark, as per cent. sugar per 1 cm., then knowledge of the average value of k or $1/k$ will enable us, from the rate of movement observed in the present case to calculate the expected gradient. The mean value of $1/k$ for the nine determinations in our previous work was 0.179. Calculation of the gradient expected in the present experiment is set out below. For simplicity only the normal downward movement will be considered.

Mean cross sectional area of sieve-tubes per stem	= 0.128 sq. cm.
Mean amount of carbohydrate moved per stem	= 1.678 gm.
Mean time	= 51.21 hours.
Rate per 1 cm. ² of sieve-tube per 1 hour (R)	= 0.256 gm.
∴ Expected mean gradient of total sugars in the whole bark	= 0.256×0.179 cm. = 0.0458 percent. per 1 cm.
Final gradient observed (difference between upper and lower regions) in the whole bark	= 0.834 per cent. sugar.
Distance between midpoints of the two regions	= 27.33 cm.
∴ Final gradient observed	= 0.0305 percent. per 1 cm.

It will be seen that the final gradient observed is 66 per cent. of the mean gradient expected, so that unless the mean gradient during the experiment was less than the final gradient, which seems unlikely, then the rate of transport of sugar for unit gradient in this experiment was not widely different from the rates previously recorded.

SECTION 4. DISCUSSION.

The ideas underlying these experiments on cessation of movement and reversal of movement have been fully discussed in presenting the data, and little further comment is required. It may be convenient, however, to recapitulate the main features of the argument.

The work reported is a study of the changes in gradient associated with changes in movement. It is assumed that the vertical gradients observed may be analysed into two components—a dynamic gradient, of translocatory material, which alters as the rate or direction of movement alters, and a static gradient of storage material, which is relatively constant. The first experiment, on cessation of movement, establishes the existence of negative static gradients in nitrogen compounds, for when transport comes to a standstill the vertical gradient in sugar concentration becomes zero, but there still remains a marked negative gradient in total N and total crystalloid N. Where normal downward movement is still proceeding the positive dynamic gradient is masked by the negative storage gradient, but the net negative gradient is less than where transport has ceased. The difference is an estimate of the dynamic gradient.

In the second experiment the direction of movement between two regions of stem, Upper and Lower, is reversed by reversing the relative positions of 'source' and 'sink'. Reversal of the normal direction of movement is accompanied by a reversal of the gradient of total sugars, and a steepening of the originally negative gradient of nitrogen compounds. This change in the nitrogen gradients is interpreted as the reversal of an initially positive dynamic gradient, superimposed on a relatively constant static gradient. The fact that these changes take place mainly in the inner half of the bark suggests that the dynamic gradient is localized in the sieve-tubes while the static gradient is largely a phenomenon of storage in rays and cortex.

An approximate estimate of the total dynamic gradient is obtained by subtracting the sum of the concentrations in the receiving regions from the sum of the concentrations in the supplying regions. The static gradient between Upper and Lower regions is thereby eliminated, for the supplying regions are an Upper and a Lower region, and the receiving regions are equally an Upper and a Lower region. The total dynamic gradient so calculated is compared with the total movement observed. Judging from the relation between the sugar gradient and the rate of movement of carbohydrates, the dynamic gradients in total N, protein N and amino-acid N would be adequate for the total amount of nitrogen transport observed. This suggests that the acceleration mechanism in the sieve-tubes acts impartially on sugars and nitrogen compounds.

Although further experiments of this type will be required before these

conclusions can be fully established, the conception that the gradients observed in the plant may have both dynamic and static components does seem to provide a useful tool for the analysis and interpretation of transport phenomena. The conception is admittedly formal and, until the static and dynamic components can be identified by physico-chemical or spatial analysis of the gradients, must remain a point of view rather than an explanation. From the data available it cannot be said, of any of the chemical groups into which total N was subdivided, that the group is wholly dynamic or wholly static. The two fractions which contribute most to the dynamic gradient in the reversal experiment, viz. protein N and amino-acid N, contribute also to the negative static gradient. Asparagine, on the other hand, contributes considerably to the static gradient, but has also a small dynamic component. It is possible, of course, that not all the protein N, amino-acid N or asparagine N is in the same physical state in the plant, and the distinction between dynamic and static gradients may be physico-chemical. Adsorption relationships may be involved. Spatial distribution appears, however, to offer a more promising clue, for the reversal of the dynamic gradient is mainly a phenomenon of the inner half of the bark, which contains most of the sieve-tubes. A large part of the static component in the bark is almost certainly due to vertical storage gradients in rays and cortex. If only rays and cortex are involved it may be possible, by more extensive radial subdivision of the bark, to separate static and dynamic components, and to establish the existence in the sieve-tube tissue groups of a positive gradient in the direction of movement. If, however, there are vertical storage gradients in the companion cells as well, then radial subdivision will not effectively separate the static and dynamic components, for companion cells and sieve-tubes have the same radial distribution. The possibility must also be faced that there is a storage or static component in the sieve-tubes themselves.

5. SUMMARY.

(1) When movement of nitrogen down a stem is brought to a standstill by removal of the leaves and ringing the stem close to the ground, there still remains a marked negative gradient in protein N and crystalloid N in the bark. On the other hand, when the movement of carbohydrates is similarly brought to a standstill, the sugar gradient disappears. Thus while zero movement of nitrogen is associated with a negative gradient in the bark, zero movement of carbohydrates is associated with zero gradient.

(2) Where downward movement of nitrogen is still proceeding, the negative gradients in protein N and crystalloid N are smaller than where downward movement is brought to a standstill.

(3) It is suggested that the negative gradient in nitrogen observed in

the bark of the normal plant consists of two components, a negative static gradient of storage nitrogen which persists after movement ceases, and a positive dynamic gradient of translocatory nitrogen, which disappears when movement ceases.

(4) Reversal of the normal downward direction of movement of carbohydrates and of nitrogen is accompanied by a reversal of the gradient of sugars and a steepening of the originally negative gradient of nitrogen compounds. This change in the nitrogen gradient is interpreted as the reversal of an originally positive dynamic gradient, superimposed on a relatively constant static gradient which is negative. That these changes in gradients are almost entirely confined to the inner half of the bark supports the suggestion that the dynamic gradient is in the sieve-tubes, and the static gradient is mainly due to nitrogen stored in the other tissues of the bark.

(5) The total dynamic gradient is calculated by means of a simple approximate formula and compared with the total movement of nitrogen. Judging from the observed relation between gradient and movement of carbohydrates, the dynamic gradient in total N, protein N, and amino-acid N would be adequate for the amount of nitrogen movement observed.

(6) This suggests (*a*) that the acceleration mechanism in the sieve-tubes acts impartially on sugars and nitrogen compounds and (*b*) that either total N, protein N, or amino-acid N may be associated with longitudinal movement. It is pointed out that the presence of sieve-pores should render the movement of colloids possible, and that if colloids in solution are moved, then crystalloids of every kind should also take part in translocation.

LITERATURE CITED.

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4. —————: The Factors Determining the Rate and the Direction of Movement of Sugars. *Ibid.*, 571-636, 1928.
5. CZAPEK, F.: *Biochemie der Pflanzen*. Bd. ii. 296, 1925.

A Monograph of *Stigmaria Bacupensis*, Scott et Lang.

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With Plates I-VII.

INTRODUCTION.

THE purpose of these investigations is the complete study of the general anatomy of the main axis of *Stigmaria bacupensis*, Scott et Lang, and its appendages.

Dr. D. H. Scott was the first who noted the characteristic features of the plant and named the new species. He gave a short account of this type in the catalogue of his splendid collection in the British Museum (Natural History), but never studied or figured it. In the Belgian Coal-balls included in Buxharmont beds,¹ I found a considerable number of specimens of *S. bacupensis*, some of them being beautifully preserved.

I have to express my thanks to Dr. Scott for his permission to study a plant that he was the first to discover. He also obtained from the British Museum permission for two slides of his collection, nos. 950 and 1773, to be figured in my paper as 'type' specimens (Pl. I, Figs. 1 and 2).

If Dr. Scott made the species, Professor W. H. Lang, though he did not describe it, was the first to figure a *S. bacupensis* under its true name. For that reason, according to the Vienna rules the author of the manuscript name and the author who first published it, must be cited together. Hence the species becomes, *S. bacupensis*, Scott et Lang.

As far as I know specimens of *S. bacupensis* have been already figured by Williamson (17-18), Weiss (15), Gothan (1), Lang (5), Leclercq (16), and Koopmans (4), as *Stigmaria ficoides*, Brong., or without determination, or under its true name. None of them have described in detail either the very characteristic features of the main axis or the appendages, as will be seen in the historical survey.

This species occurs in the English Lower Coal-Measures; it has been

¹ Buxharmont beds correspond to the English Lower Coal-Measures. See Leclercq (6).

found in various localities: Dulesgate, Halifax, Shore Little Borough, Bacup, and Sharneyford. It has been observed in other countries also: in Belgium in Bouxharmont beds (6); in the Netherlands in Finefrau Nebenk seam (4); in Germany from Katharina horizon (1). However this species seems to be most frequently found in England and in Belgium.

I am indebted to Professor Weiss of the University of Manchester and to Professor Seeward of Cambridge for the kind reception I met with during my stay in these two Universities. I also express my thanks to Mr. Edwards of the British Museum who gave me all possible means of examining the Scott and Williamson collections, I must also especially thank Professor C. Fraipont of the University of Liège for all his kind assistance.

HISTORICAL SURVEY.

In 1881, Williamson (17), for the first time, figured a transverse section of a rootlet of *S. bacupensis* which he attributes to a very young root of *S. ficoides*, Brong. He does not draw special attention to the peculiar anatomy of the appendage. He considers the empty space opposite the narrow parenchymatous band connecting the bundle with the outer cortex, as an accident. (See Pl. I, Fig. 3, of this paper where Williamson's rootlet is re-figured.) He says, p. 292: 'In the specimen figured, some of the cells of the outer zone have become partially detached from the outer ring' This is not quite true. In reality no tissue has been *detached*; the empty space is the result of the *decay* of a larger-celled tissue characteristic of the rootlet (Pl. VI, Figs. 46 and 47), as can be seen in Pl. I, Fig. 4 where a Belgian specimen in the same state of preservation is represented.

Williamson, however, notes the small dimension of the rootlet; he observes, p. 292 'that the diameter of the entire young rootlet is less than that of the central inner bark cylinder of a matured rootlet' of *S. ficoides*, Brong. The small dimension of the rootlet is a particular feature of the *S. bacupensis* appendages as will be seen further below.

In 1887, Williamson's (18) 'Monograph on *S. ficoides*' appeared. The author figures (Pl. IX, Fig. 18) a transverse section of 'an extremely young root of *Stigmara* and considers it as a small extremity of true root' (p. 15 and 42).

I am inclined to consider Williamson's specimen as a young axis of *S. bacupensis*, Scott et Lang. The disturbed vascular cylinder, the great number of rootlets and their peculiar structure, suggest it. As I had examined the specimen in Williamson's figure only I could not be certain of my determination, so I asked Professor Weiss to clear up this point. He wrote me that he had also noted the resemblance of Williamson's figure to *S. bacupensis*, and that he examined the original preparation when he was

at the British Museum. 'The specimen', he says, 'shows some central remains in the pith but no spiral elements are to be seen. The tissues are not sufficiently preserved. Still I think it was very probably a *S. bacupensis*, though it cannot be determined with certainty.'

The presence of spiral elements in the centre of the main axis is one of the characteristic features of *S. bacupensis*, so if they do not occur in Williamson's specimen, the latter is certainly not a *S. bacupensis*. However it might have happened that the delicate spiral elements have become disorganized. In any case the determination is doubtful.

From 1895 to 1923 Dr. D. H. Scott (9) wrote his Catalogue as his collection of slides grew. He gave the following short description of the species: 'Rootlets appear more numerous than in *S. ficoides*, and medullary ray bigger in proportion to bundle of rootlet . . . Stele cut obliquely, shows probably primary tracheides in pith. . . . The middle is almost solid primary wood with small barred tracheides. Periderm and bases of numerous rootlets shown.'

This short description is of course very incomplete. Dr. Scott has noticed neither the peculiar structure of the outer cortex of the main axis nor the peculiarities of the appendages which define the type. These features are particularly well shown in Belgian specimens.

In 1902, Professor Weiss (15) published a very interesting paper on 'The vascular branches of Stigmarian Rootlets'. He investigated rootlets showing fine vascular strands starting out from the protoxylem of the bundle, and running out through the middle cortex to terminate in the outer cortex in connexion with an extensive patch of tracheidal tissue. His study is founded on the examination of *S. ficoides* rootlets and on 'a type not uncommon among stigmarian rootlets', as he correctly noted 'that a careful examination of a large number of rootlets of this type . . . convinces' him 'that it is a somewhat different type of rootlet'. The rootlet figured by Weiss (Pl. XXVI, Fig. 3) is a *S. bacupensis* appendage. A close comparison can be established between these figures (repeated in this paper Pl. I, Fig. 5) and the type specimen (Pl. VI, Figs. 46 and 47). It will be shown that Weiss's undetermined rootlet possesses the characteristic features of a typical *S. bacupensis* appendage (see p. 49 the *S. bacupensis* rootlet diagnosis).

In 1923 M. W. Gothan (1) published a study on the 'Karbon und Perm Pflanzen'. He figures (Pl. XLIII, Fig. 4) a group of rootlets that he determines as stigmarian appendages. The monarch structure of the bundles, its connexion with the outer cortex by a strand of parenchymatous tissue, and the diameter of the rootlets are characteristic features of *S. bacupensis* rootlets.

The comparison between Gothan's appendages group and the group of *S. bacupensis* appendages (Pl. VI, Fig. 47) shows the identity of the rootlets. In the same year, Professor W. H. Lang (5) gave a very interesting account

‘On the apparently endogenous Insertion of the Roots of *Stigmaria*’. His investigations are founded on the examination of a young axis of *S. bacupensis*. His researches concern the relation of the rootlets to the outer tissue of the Stigmarian axis. He does not describe the species, but for the first time, as it has been noted in the introduction, he figures that species with its true name.

Two years later (1925) I published a paper (6) in which I pointed out the presence of two *S. bacupensis* specimens in the coal-balls of Buxharmont. The first one, figured on Pl. XXVIII, Fig. 5, is a young axis magnified (thirty times) to show on the photograph the numerous rootlets, cut in various directions, which surround it. A transverse section made through that specimen is figured in this paper (Pl. I, Fig. 6); the second specimen figured on Pl. XXVII, Fig. 3, of my paper of 1925, is a rather stout axis. The stele alone is preserved and shown magnified only ten times. This specimen named *Stigmaria cf. bacupensis* is not correctly determined. If the secondary wood is broken down in numerous narrow bundles as is usual in *S. bacupensis*, the primary wood lay here at the inner end of each wedge. It is formed by smaller and not very regular elements disposed in a centrifugal manner such as *S. ficoides*. The distinction between primary and secondary xylem is not sharp as in *S. bacupensis* (Pl. II, Fig. 9). The centre of the stele is hollow; it has been certainly occupied by a fair-sized pith which is not preserved, and is replaced by a Stigmarian appendage running over. No traces of spiral elements belonging to the primary wood can be seen in the centre, as always occur in *S. bacupensis* axis.

For that reason I will call that specimen *Stigmaria* sp. till I have found another specimen surrounded by a cortex.

In 1927 Dr. M. Hirmer¹ (2) published his ‘Handbuch der Paläobotanik’, in which he re-figures (p. 294, Fig. 346) Gothan’s undetermined group of Stigmarian appendages and names them *Stigmaria ficoides*, Brong. For the reasons stated above, I consider these appendages as belonging to *S. bacupensis*. In the same paper M. Hirmer gives (p. 296) a short account of *S. bacupensis*; he says: ‘*St. bacupensis*, Scott. Lower Coal-Measures of England and Belgium. In transverse section the primary xylem consists of very numerous and small elements. Rootlets of evident endogenous formation.’

In 1928 appeared R. G. Koopmans ‘Researches on the flora of the coal-balls from the “Finefrau Nebenbank” Horizon in the province of Limbourg (the Netherlands).’ The author (4) figures (Pl. XII, Fig. 93) a transverse section of two steles without cortex, of *St. cf. bacupensis*, Scott. He considers it possible that these specimens belong to that later

¹ Hirmer, M.: *St. bacupensis*, Scott. Lower Coal-Measures von England und Belgien. Primary xylem Partien im Querschliff sehr schmal und zahlreich. Wurzeln offenbar von endogener Entstehung (p. 296).

species, though his determination is only founded on the examination of the figures published in my paper, 'Les coal balls de la couche Bouxharmont des charbonnages de Werister'. His determination seems to be correct.

DESCRIPTION OF *STIGMARIA BACUPENSIS*, SCOTT ET LANG.

Stigmaria bacupensis.

1895-1923: Scott, Manuscript.

1923: Lang, Mém. Procéd. Manch. Litt. Phil. Soc., vol. lxxvii, Part II, p. 101, Pl. II, Fig. 1.

1925: Lœclercq, S., Mém. in 4° Soc. Géol. de Belg., tom. vi, p. 47, Pl. XXVIII, Fig. 5.

1928: Koopmans, R. G., Géol. Ber. voor het Néderl. Myn., i, p. 17, Pl. XII, Fig. 93.

Stigmaria ficoides.

1881: Williamson. Royal Society. On the Organization of Fossil Plants, Part XI, p. 292, Pl. LIII, Fig. 16.

1887: Williamson, Monograph on *S. ficoides*, Paleontograph. Soc., pp. 15 and 42, Pl. IX, Fig. 18.

1927: Hirmer, Handbuch der Paläobotanik, München und Berlin, p. 294, Fig. 346.

Stigmaria, sp.

1902: Weiss, F. E., Ann. Bot., p. 565, Pl. XXVI, Figs. 3 and 4.

1923: Gothan, W., Leitfossilien, Berlin, p. 156, Pl. XLIII, Fig. 4.

ANATOMICAL STRUCTURE.

As already mentioned, the species *S. bacupensis* was made by D. H. Scott as the result of the examination of English specimens now in the British Museum. I have shown in this paper as type specimens (Pl. I, Figs. 1 and 2), two examples nos. 950 and 1773 borrowed from the Scott collection, though the whole study of the species is based largely on the examination of Belgian specimens found in coal-balls of the Bouxharmont beds. The Belgian coal-balls examined are nos. 2, 624, 678, 802, 724. They have supplied very numerous preparations. For ease of text transcription, the specimens will be designated by the number of the preparation in which they are found. For instance when it is said that in no. 624, the glandular zone of the outer tissues of the main axis is the most striking, it means that in the specimen of *S. bacupensis* included in preparation

no. 624, the glandular zone of the outer tissues of the main axis is the most clearly seen.

We have now to consider the internal structure of the main axis (vascular cylinder and cortex) and its appendages.

THE MAIN AXIS.

The dimensions of the whole axis (stele and bark), vary according to the age of the specimens. In apparently well-developed specimens (like nos. 624 and 678; Pl. I, Figs. 7 and 8), the bark must have had a circumference of 25–35 mm. while the central cylinder diameter is 3–4 mm. only.

(a) VASCULAR CYLINDER.

The primary wood. One of the most striking features of the anatomy of *S. bacupensis* is the structure of the primary wood of the vascular cylinder. This differs in essence from all the other species of *Stigmaria* in being neither centrifugal nor centripetal in its development. Towards the centre of the axis, the secondary tracheides become smaller without losing anything of the regularity of their arrangement (Pl. II, Fig. 9) as will be seen below. Then within the cylinder of secondary wood we come to a very definite primary wood beginning with small spiral elements on the outside (Pl. II, Fig. 9) followed on the inside by a tissue consisting of numerous rows of spiral elements and small barred tracheides of various dimensions (Pl. I, Fig. 1; Pl. II, Fig. 10). No pith is present. The transverse sections (Pl. II, Fig. 10) show clearly that within the small elements of the periphery, as we advance further inwards, elements of various size are mixed. Some of them are twice the size or more of the peripheral elements, others are as small as these or still smaller. This fact is well illustrated in the specimen 624 (Pl. II, Fig. 11) where a rather wide element, situated in the centre of the axis is surrounded by seven smaller elements (Pl. II, Fig. 11) and also in specimen 624, 1–7 (Pl. I, Fig. 12) where a row of elements of various dimensions joins the ring of secondary wood.

Beyond that, specimen 624 only shows in a transverse section, wide elements with reticulate thickening (Pl. II, Fig. 13) like the characteristic reticulate tracheides of *Lepidodendron selaginoides*, Stern. No ring forming a broad continuous zone of primary wood can be found, and as Dr. Scott says in his catalogue 'The middle (of the axis) is almost solid primary wood, with small barred tracheides'.

The elements of primary wood being thin walled and of small zone do not offer a great resistance to the intrusion of the stigmarian appendages; these latter, very frequently, run over the centre of the axis, crushing the primary xylem (Pl. I, Fig. 8). For that reason good radial sections are rare. However, the radial section (Pl. III, Fig. 14) shows very narrow

elements at the outside of the wood exhibiting spiral, annular, and barred marking (Pl. III, Figs. 14 and 15). Further inwards, the wood consists of elements showing the same thickening but of various dimensions (Pl. III, Figs. 14, 15, and 16). It is not possible to say if the primary wood consists in protoxylem and metaxylem; the differentiation and the direction of the woody elements in which they are developed are not clear enough.

In three radial sections made through the woody cylinder of two Belgian specimens, nos. 624 and 678, we have found mixed with the primary wood elements, a thick bearded mycelium (Pl. III, Fig. 17). The same fungus has been met with among the primary wood of one of the two English specimens (no. 1773) figured in this paper (Pl. I, Fig. 2). Though four other specimens have been investigated to see if the association of the two plants was constant, we are not yet able to assert it with certainty. It is very difficult to obtain good and clear radial sections through the delicate primary wood whose elements are generally crushed by intruded Stigmarian appendages. Yet the presence of the same fungus in three well-developed main axes seems to remove the possibility of an accidental invasion. It must be said, however, that young axes appear not to have been invaded.

The secondary wood is generally well preserved and of normal type. It consists of a well-developed vascular cylinder divided up into numerous narrow bundles by the numerous principal medullary rays (Pl. I, Figs. 1 and 8; Pl. II, Fig. 9). The bundles consist of radially arranged scalariform tracheae showing only slight irregularity where new rays of wood elements become added, as secondary growth proceeds. The tracheides are of medium size, and square in transverse section. Towards the inner margin, though the distinction between primary and secondary xylem is sharp, the inner end of each wedge is loose. In addition to the principal rays, numerous narrow secondary rays traverse the wood; their thinner tissue has undergone some disorganization so that they appear on the transverse section more like long gaps (Pl. I, Fig. 1; Pl. II, Fig. 9).

The secondary xylem tracheae present scalariform pits on both radial and tangential walls (Pl. III, Figs. 18 and 19) and show the spaces between the transverse bars bridged across by fine threads as has been noted and figured by Williamson for *Lepidodendron mundum* as well as in the tracheae of other *Lepidodendra*. The numerous parenchymatous rays, generally one cell thick and one to forty cells in height, can readily be seen on the tangential longitudinal section (Pl. III, Fig. 18). The radial sections show clearly the secondary tracheides becoming smaller from the outside to the inside and joining closely the narrow spiral elements of the primary wood (Pl. III, Figs. 15 and 19). No pith is present. A feature which distinguishes this type of *Stigmaria* from the *Stigmariae* with a solid primary wood, is the woody cylinder broken up into numerous and narrow distinct wedges. The frequent presence of broad medullary rays traversed by the

appendages bundles split up the woody ring. The tangential section shows (Pl. III, Fig. 18) the narrow bundles anastomosed laterally with each other, forming a network, with numerous meshes in which medullary rays and the appendages bundles are enclosed. Radial sections show the secondary wood very divided for the same reason (Pl. III, Fig. 19 ; Pl. V, Fig. 37).

In conclusion, I should like to mention the characteristic features of the vascular cylinder : *Solid primary xylem with small spiral, annular, and barred-marking elements of various size. No evident direction in the development ; no pith. Secondary xylem broken into numerous narrow bundles, the inner end of these latter is loose ; numerous medullary rays.* The delicate tissue of the cambium and the phloem has not been observed in the preparations examined.

(b) THE CORTEX.

From the outer tissues, the outer cortex is generally well preserved. Remains of inner and middle cortex are rare. The space left by the decay of these soft tissues is usually largely invaded by stigmarian appendages (Pl. I, Fig. 8 ; Pl. II, Fig. 9).

In the outer cortex, it is possible to distinguish four zones. These are from within outwards (1) an irregular periderm (Pl. III, Fig. 20) ; (2) a zone with dark-coloured group and tangentially elongated parenchymatous cells (Pl. II, Fig. 9) ; (3) a clear zone of tissue made up of moderate sized cells which may show indications of tangential and radial divisions (Pl. II, Fig. 9) ; (4) more or less complete remains of an outermost zone of very small-celled tissue (Pl. I, Fig. 6 ; Pl. V, Fig. 36).

The *periderm* consists of a prosenchymatous tissue formed by cells with parallel sides and pointed ends, mixed with wide-celled files (Pl. IV, Fig. 22).

In transverse section, the prosenchymatous cells are radially elongated except the inner-cells which are more tangentially extended (Pl. IV, Fig. 21).

Their layers, generally arranged in definite vertical files, are separated throughout the width of the periderm by a wide-celled portion (Pl. III, Fig. 20), and against the dark-coloured group of zone II (Pl. II, Fig. 9 ; Pl. III, Fig. 20).

Sometimes files do not quite reach to the outer border of the secondary tissue and taper away before reaching the outer margin (Pl. III, Fig. 20).

Very probably in some cases short files may be produced by subsequent division of the cells of the periderm. The number of the layers increases with the age of the specimens.

The wide-celled files, typical of stigmarian periderm are rather frequent in *S. bacupensis*. In well-developed periderm formation, they form lighter lenticular areas enclosed by dark prosenchyma files (Pl. III, Fig. 20 ; Pl. IV, Fig. 21).

In younger axes they appear rather wedge-shaped in transverse section, increasing in size towards the periphery or the inner margin (Pl. II, Fig. 9). They are formed among the ordinary radial files by a certain number of the cells being left thin-walled and becoming extended in the tangential direction. They have a great power of division and show numerous radial septa (Pl. III, Fig. 20; Pl. IV, Fig. 21).

It is in the tangential section that the heterogeneous nature of the cells of the periderm is the most striking. The prosenchymatous cells are as already mentioned, narrow parallel-sided and with pointed ends (Pl. IV, Figs. 22 and 23). They are commonly narrower towards the inside where they constitute the largest portion of the periderm. The thin-walled wide cells occur rather in definite vertical files than in lenticular areas (Pl. IV, Fig. 22). They are divided by horizontal septa into chambered-cells, and each segment may undergo further vertical division, so that later, the outline of the original extended prosenchymatous cells is lost (3, p. 306) (Pl. IV, Fig. 23), but sometimes the occurrence of the pointed end-segments still gives the clue of the origin of the mesh. Sometimes very simple chambered-cells are met with which consist of cells with a few horizontal septa.

In radial section the prosenchymatous cells consist of fairly straight rows of tissue, narrower towards the interior (Pl. IV, Figs. 24 and 25). The chambered-cells with their numerous daughter-cells form a large conspicuous parenchymatous strand surrounded by dark prosenchymatous formation (Pl. IV, Fig. 25).

We will now consider in which direction the periderm has been formed. In none of the specimens we have investigated, have we found the phellogen preserved, but it has been already mentioned, in transverse sections, toward the interior, the prosenchymatous cells are more tangentially extended (Pl. III, Fig. 20; Pl. IV, Fig. 21), that is to say, that recently formed tangential walls have occurred and probably represent an early stage in the development of periderm.

In radial section made through the periderm formation the straight lines tissue is narrower towards the inner margin (Pl. IV, Figs. 24 and 25). For these reasons I am inclined to determine the position of the phellogen near the inner margin, and to consider the periderm development as centripetal.

In her paper on the 'Physiological anatomy of the periderm fossil Lycopodiale' Kisch (3, p. 294) noted in *Stigmariae* two distinct types at least in regard to the position of the phellogen. In the case of irregular periderm with wide-celled outer portion, she locates the phellogen in the middle of the periderm formation just at the junction of the regular radially disposed cells, and the irregular wide-celled outer portion.

'In this type,' she says, 'though a disorganized band of cells is all that can generally be distinguished, the phellogen is probably at the junction of

the two different kinds of tissue and forms the wide-cells to the exterior and the narrower files on its inner surface by means of further radial divisions.'

I do not agree with this view in the case of *S. bacupensis*. In all the specimens of that species I have examined (and some of them have perfectly preserved periderm) I never found in the middle of the periderm formation either meristematic cells or a disorganized band of cells which may be distinguished as phellogen. If the periderm is of an irregular formation a sharp distinction between a wide-celled *outer portion* and a regular radially disposed cell *inner portion* does not occur in *S. bacupensis*. In transverse section, the chambered-cells of the somewhat lenticular areas run the whole width of the periderm. If their maximum width most frequently occurs towards the exterior, the extremity of the decreasing lenticular strand also reaches the inner margin (Pl. II, Fig. 9; Pl. III, Fig. 20; Pl. IV, Fig. 21).

The irregular periderm formation of *S. bacupensis* is quite easy to explain when the phellogen is situated towards the inner margin. In very young specimens, regular prosenchymatous tissue only is formed on the outer side of the phellogen. A little later, in a somewhat older example some cells are left thin-walled by meristematic cells and are transformed into chambered-cells (wedge-shaped and afterwards lenticular area transverse section). In very old specimens, the increasing girth of the axis forces the gradual increase of the outer tissues. This enlargement is assured by the multiple divisions of the chambered-cells which have such a great power of division that they may lose their original outline. In such old examples, where periderm formation is well developed, a regular inner portion in which no wide-cells are found, may occur. The chambered-cells have been located towards the middle and the exterior periderm up to the point where the increasing girth of the axis results in numerous divisions of the outer tissue to keep pace with its enlargement.

To sum up the characteristic of the first zone of the outer cortex it may be said that *the periderm is of an irregular type consisting of normal prosenchymatous tissue mixed with wide-celled portions. The position of the phellogen is on the inner margin, consequently the periderm is developed in a centripetal direction.*

The *second zone* of the outer cortex is equivalent to the band of cells characterized by dark contents noted in the outer tissues of *Stigmariae*. This zone in *S. bacupensis* is highly specialized, and one of the most striking features of the species.

In transverse section, at fairly regular intervals, groups with dark contents appear; these are generally distributed in a single row, though two groups may occur on the same ray (Pl. I, Fig. 8; Pl. II, Fig. 9).

They consist mainly of circular or oval areas (Pl. IV, Figs. 26 and 27),

whose diameter is generally uniform, though groups of various sizes occur. They are connected by a wide-celled parenchyma tangentially, and sometimes radially extended (Pl. II, Fig. 9; Pl. IV, Figs. 26 and 27) which shows few horizontal or vertical secondary septa. Just opposite the rootlet's base the dark group is missing, and a dark small-celled tissue with thick walls fills its place (Pl. I, Fig. 6; Pl. II, Fig. 9).

Of all the specimens which have been investigated, no. 624 shows the most highly specialized glandular parenchyma (Pl. I, Fig. 7; Pl. II, Fig. 9). In a small axis like number 2 (Pl. I, Fig. 6) dark groups are rare; they are just beginning to develop. In the example with well-developed periderm, the parenchyma connecting the dark areas is more or less crushed (Pl. I, Fig. 8; Pl. III, Fig. 20). The nature of the content of the group is not easy to determine. It cannot, however, be interpreted either as sclerotic nest or as imperfectly preserved fungal hyphae for, in transverse section as well as in radial and tangential, the groups are filled up by black bodies which may be remains of cell-wall and products of secretion (Pl. IV, Figs. 26 and 27). On the other hand, some of the glandular strands seem to have a somewhat central reservoir (Pl. IV, Fig. 27). The formation of such a passage is shown in a young branched axis. In a transverse section, a glandular strand in formation shows one of its cells which has been divided in two by a radial septa (Pl. IV, Fig. 28). In the width of that septa a lenticular intercellular space, bordered by two cells, is formed; the whole structure is like a stomata. This pseudo-stomata is the first indication of a central passage. The adjoining cells probably underwent further analogous divisions, and constituted a definite strand of secretory cells. Yet disorganization is so advanced in glandular strands which have reached full development, that no peripheral layer of glandular cells occurs with certainty.

However, if the slides I have examined have not shown very young axes in which it would have been interesting to follow the glandular sacs development, Professor Lang (5), as already mentioned, has figured a portion of the outer cortex of a small axis of *S. bacupensis*, in which early stages of glandular sac development are seen. Prof. Lang distinguishes three zones in the outer cortex. His zone 'a' is equivalent to our zone II. He says, 'the zone "a" consists of cells, the walls of which are often dark, and which may show indications of tangential divisions'. The zone 'a' is shown in Pl. II, Fig. 5 of his work. In the right part of the figure, a small black mass occurs; eight parenchymatous cells are disposed around it (see Pl. IV, Fig. 29). On the left of the figure a second black mass is seen, in the middle of which occurs a somewhat light spot, which may represent an early stage of the formation of the central canal. The dark substance is surrounded by six large cells; one of these seem to be just divided by a radial septa.

I am inclined to consider the whole as a different stage of glandular strand development. As is known, in order to form a glandular strand, all the cells of a vertical row divide themselves into four cells by two perpendicular divisions. Between the four resulting daughter-cells an intercellular space occurs, which increases in size by further radial and tangential divisions, undergone by the adjoining cells. The surrounding cells of the dark mass of Lang's photograph would be this layer of glandular cells, disorganized, in mature axes.

If the presence of cells showing signs of recent divisions is unusual in secretory tissue, it is normal to meet them in quite immature secretory sacs, and we know that Lang's specimen is a very young axis.

The investigations we have carried out show that the secretory sacs seem to have a schizogenous development; they belong to the primary cortex.

The tangential section shows that the secretory sacs do not form either an anastomosing system or continuous large ducts, but consist of isolated sacs of various lengths and width, rarely branched, and ending blindly (Pl. V, Fig. 30). Their distribution in the surrounding parenchyma is very irregular. Pl. V, Figs. 31, 32, and 33 show various aspects of the secretory sacs. Some of them (Pl. V, Figs. 31, and 32) are wide lenticular areas connected to one another by two rows of large cells elongated tangentially. Some others are long and narrow (Pl. V, Fig. 33), or short and narrow (Pl. V, Fig. 30), but all are surrounded on both sides by wide cells tangentially elongated, which distinguish them from the well-known wide-celled portion of the periderm not being chambered in numerous secondary cells.

Sometimes a group of small cells is located within the secretory sacs (Pl. V, Fig. 34); this explains the group of small cells without dark contents found in the transverse section in the place of secretory areas.

The presence of a divided secretory strand explains also the double rays of the group and the narrow canals observed in the transverse section. Whatever shape they may have, the secretory sacs are occupied by dark bodies or rods of various size and form, which may be the remains of dissolving cell-walls mixed with waste product (Pl. IV, Figs. 26 and 27; Pl. V, Fig. 31).

Generally the secretory sacs show a diffuse central passage (Pl. IV, Fig. 27; Pl. V, Figs. 31, 32, 33, and 34), and remains of periphery glandular cells, but these latter appear either disorganized or like two black layers surrounding the canal. The radial section confirms the somewhat lenticular aspect of the secretory sacs observed in the tangential section; Pl. IV, Fig. 24 and Pl. V, Fig. 34 show some of them cut radially. In the magnified glandular strand (Pl. V, Fig. 34) we see the dark content in the diffuse central passage, and the dark peripheral layer of glandular cells. This latter joins, on the inner side, the straight line tissue of the periderm which abuts

against it, and on the outer side, a wide-celled parenchyma elongated radially, well seen in transverse section (Pl. IV, Figs. 26 and 27); without and within are narrow thick-walled cells.

Where the sections show the point where the periderm touches the secretory sacs, these latter are surrounded on both sides by parenchyma elongated radially.

On the whole *the second zone is characterized by glandular strands (sacs) of schizogenous formation, in which the central passage is occupied by remains of cell-walls and waste-products. In transverse section they are disposed at fairly regular intervals in a single circle. They consist of circular or oval areas generally connected by a wide-celled parenchyma, tangentially, and sometimes radially extended.*

In tangential and radial sections they consist of isolated sacs of various length and width, rarely branched, and ending blindly. Their distribution in the surrounding parenchyma is irregular.

It is not the first time that glandular strands have been noted in Stigmarian cortex; Professor Weiss (14) has described a secretory strand in *Stigmaria radiculosa*, Hick. This secretory strand was exactly like those found in a corresponding position in *Lepidodendron fuliginosum*, Will.

Zones III and IV. The two later zones of the outer cortex present no particular feature.

In transverse, tangential, and radial sections (Pl. II, Fig. 9; Pl. IV, Fig. 24; Pl. VI, Fig. 44) zone III shows a parenchymatous tissue with an average breadth of eight to twelve rows of moderate sized cells, which may undergo further tangential and radial divisions (Pl. V, Fig. 35).

The outermost zone (IV) is rather defective. It consists of somewhat broken-down small-celled tissues of ten to twelve layers in thickness (Pl. V, Fig. 36; Pl. VII, Fig. 54).

This soft parenchyma, usually crushed or broken by the appendages, is left ill preserved in most of the preparations (Pl. VI, Fig. 44; Pl. VII, Fig. 54).

In conclusion, I should like to sum up the characteristic features of the outer tissues of *S. bacupensis*.

The outer cortex includes four zones:

- I. A periderm centripetally developed consisting of normal pro-senchyma mixed with chambered-cells.
- II. A particular zone of isolated schizogenous glandular strands whose shapes vary in length and width, and end blindly.
- III. A parenchymatous tissue made up of moderate-sized cells which show power of division.
- IV. A delicate small-celled tissue generally broken down.

THE APPENDAGES OR ROOTLETS.

The above account has described the anatomy of the whole axis; it remains now to describe the appendages.

The relation of these latter to the main axis will be considered first, and afterwards their characteristic structure when they are free, will be investigated. The true morphological nature of the appendages is still unknown, but, for facility of text transcription the figurative term 'rootlets' will be used.

By means of radial sections made through the whole principal axis, the rootlet's bundle-course is readily followed. The vascular strands start from the outer margin of the primary wood carrying with them some protoxylem elements. They bend sharply outwards and pass horizontally through the whole width of the secondary wood (Pl. V, Fig. 37), splitting up the woody cylinder in distinct wedges (Pl. I, Figs. 1 and 6; Pl. II, Fig. 9). This horizontal course of the rootlet bundle through the secondary wood is usual in the *S. ficoides* appendages. On emerging from the secondary wood, they incurve bluntly (Pl. VI, Fig. 38), and pursue a nearly vertical course through the middle cortex region (Pl. VI, Fig. 39). Afterwards they enter the outer cortex horizontally, which direction is maintained through the thickness of the cortex until the base of the appendage has been reached (Pl. I, Figs. 2 and 6; Pl. II, Fig. 9).

Four different tangential sections through the woody cylinder and the outer tissue in the main axis, give an idea of the rootlet's bundle structure at various stages of its course.

The first one shows the vertically-elongated lenticular cavity of the medullary rays, in which are the wedge-shaped rootlet's bundle (Pl. III, Fig. 18). The structure of these latter agree with the characteristic tongue-shape appearance of the appendage bundle found in *Stigmara*, where the primary wood is centrifugal. The amount of secondary wood which takes part in the formation of the appendages in *S. bacupensis* is, however, less than in *S. ficoides*; it consists only of one, two, or three rows of secondary tracheides, generally connected with the secondary wood of the main axis (Pl. III, Fig. 18; Pl. VI, Figs. 40 and 40 bis.).

The second tangential section passing through the periderm shows a bundle cut transversally, consisting of a few ligneous elements, closely surrounded by the dark prosenchymatous cells (Pl. VI, Fig. 41).

In the third one, the transverse section of the rootlets increase in size (Pl. VI, Fig. 42). The bundle is surrounded by a dark ring of cell-walls which seem similar to the dark cells found in the place of the glandular strand, just opposite the base of the appendage in the second zone of the cortex of the main axis (Pl. I, Figs. 6 and 7; Pl. II, Fig. 9).

In passing through the third zone of the outer cortex of the principal

axis, a second ring, consisting of layers of thin-walled cells, encloses the inner dark circle (Pl. V, Fig. 30; Pl. VI, Fig. 43). This second lighter ring is derived from zone III of the main axis. The rootlet's cortex, from that moment, is definitely formed, for the appendages in going out, break down the last zone of the main axis cortex without taking with them some of its elements. In his paper on the roots of *Stigmaria*, Professor Lang (5, p. 104), notes that the smaller celled-tissue of the outermost zone of the main axis 'gives the impression of a layer through which the root has broken'. This is quite true. Projections of this tissue are found between the rootlet bases and crushed against their sides (Pl. VI, Fig. 44; Pl. VII, Fig. 54). In reality, the continuity of the outer cortex of the main axis with the rootlet's base tissue is only partial. Old specimens could have given the impression that the continuity was complete when their outermost zone was decorated.

It may be stated here that a particular feature of *S. bacupensis* very well seen in radial section, is the great number of appendages starting from the main axis (Pl. VI, Fig. 39), which show numerous vascular strands running obliquely through the disorganized middle cortex. In that region, the section of the rootlet's bundle is of a triangular shape, consisting of three or four rows of secondary tracheides, three or six layers in thickness, converging to the apex where the protoxylem occurs (Pl. VI, Fig. 45).

When free from the parent axis, the rootlets of *S. bacupensis* show a definite structure very uniform on the whole. Their dimensions do not vary greatly, the diameter ranging from 1 or $1\frac{1}{2}$ mm. or less. Their structure consists of an outer, middle, and inner cortex surrounding the vascular strand (Pl. VI, Fig. 46). The outer cortex, which is generally well preserved, is divided into two zones. The outer one is only two or three layers in thickness; this tissue consists of narrow and thin-walled cells which are frequently considerably larger, just opposite the protoxylem point of the bundle, than in any other part of the circumference (Pl. VI, Figs. 46 and 47). When the state of preservation is perfectly good, some cortical tracheides with wide thickening walls, are seen in that patch of large-celled parenchyma. The inner zone is generally three or five rows of cells in thickness, these cells being small in size, with thicker walls (Pl. VI, Figs. 46 and 47). Within the external zone there is usually a wide empty space, bridged by a strand of parenchyma. Occasionally at the base of the rootlet, or in rootlets situated near the main axis, some remains of the middle cortex is still found (Pl. VII, Fig. 54). It consists of one or two rows of large cells with thin walls, which extend themselves radially until their thin walls break. Pl. VI, Fig. 48 shows a rootlet still near the main axis and cut transversally. On the left of the photograph wide cells are still in connexion with the inner cortex layers; on the right, three cells have their delicate radial walls much extended and detached. Some of the rootlets

(Pl. VI, Fig. 47), though they have reached their full development, still show some of the radial walls of the disorganized wide cell.

The internal cortex forms a ring of some rows of cells enclosing the vascular strand. It consists of a few layers of delicate parenchyma connected with the outer cortex by a strand of parenchyma (Pl. VI, Figs. 46 and 47).

We shall now describe the vascular strand. In the middle cortex of the main axis, the transverse section of the bundle's appendages seems to be more important than in the free rootlet. That appearance is due to the obliquity of the bundle course through that region in the main axis. Generally, in the bundles of the free rootlet, the wood only remains, the phloem and the cambium are decayed. The xylem consists of a small strand of scalariform tracheides (eight or nine) with a somewhat triangular transverse section (Pl. VI, Figs. 46 and 47). At the more prominent angle the protoxylem is formed by three or four spirally thickened elements which generally abut against the surrounding parenchyma.

This vascular strand is monarch, and does not at all reproduce the mesarch structure characteristic of *Stigmaria* with primary centripetal wood,¹ though its main axis possesses spirally thickened elements in its centre. A noticeable feature of this *S. bacupensis* rootlet is the connexion of the bundle with the outer cortex by a strand of parenchymatous cells (Pl. VI, Figs. 46 and 47). In *S. ficoides* appendages and the rootlet probably related to *Lepidophloios fuliginosus* (14), the stele lies freely in the space left between the inner and outer cortex, by the decay of the middle cortex. The connexion of the bundle with the outer cortex is not an attribute of very young roots only, as Williamson points out (1, Pl. LIII, Fig. 16), for a large number of fully developed rootlets show this peculiar structure (Pl. I, Fig. 6; Pl. VI, Fig. 47).

In the parenchymatous bridge one, two, or three groups of spirally-marked tracheides frequently occur in close proximity to the protoxylem (Pl. VI, Fig. 46). These little strands of tracheides are the elements that Renault (Renault, B., Cours de Botanique fossile, Pl. XX, Fig. 4, Ann. des Sc. Géol. 1882, Pl. II, Fig. 8) had regarded as indicating a mode of branching distinct from the usual dichotomy. Professor F. E. Weiss (15) in 1902 has clearly investigated these structures. He confirms Renault's observations, but finds that 'these strands do not pass out to lateral rootlets as suggested by Renault, but terminate in the outer cortex, sometimes in connexion with distinct groups of large parenchymatous cells'. He points out that these fine vascular strands are given off from the protoxylem group of the bundle and pass out obliquely or horizontally, through the middle cortex, and terminate in connexion with an extensive patch of tracheidal tissue in the outer cortex.

¹ *S. weissiana*, *S. lohesti*, *S. brardi*.

The rootlet represented in Pl. VII, Figs. 49 and 49 *bis*. is cut longitudinally, and shows a delicate strand of tracheides surrounded by a layer of parenchymatous cells starting from the stele, and running obliquely through the space between the vascular bundle and the outer cortical cylinder, to the patch of large-celled parenchyma of the outermost zone of the cortex. The origin of the delicate vascular branch is unfortunately not clearly shown. The terminations of these few vascular strands in the outer cortex are not so easily observable in rootlets of *S. bacupensis* as in those of *S. ficoides*, where the outer cortex is a thin-wall tissue, much lighter than the dark-coloured zone of the outer cortex of *S. bacupensis* appendages. However, in Pl. VII, Fig. 50, a radial section passing just through the large-celled portion of the outermost cortex, shows wide spirally-marked tracheides running continuously along the rootlet. These elements are confined to those regions where the fine vascular branch terminates in the outer cortex (Pl. VII, Fig. 51).

The above description shows that the rootlets of *S. bacupensis* have numerous characteristic features which may be summed up as follows: *the monarch vascular bundle is generally connected with the outer cortex by a definite strand of parenchymatous tissue. In this latter frequently occur fine vascular strands which connect the woody elements of the bundle with the cortical tracheides. The outer cortex is divided in two zones; an inner dark one, three or five layers in thickness, and a lighter outer zone, two or three layers thick; the latter has its cells considerably enlarged just opposite the protoxylem point of the bundle where the cortical tracheides are located.* These peculiar features define plainly a type of rootlet that is of species value.

The normal type of rootlet of *S. bacupensis* may, however, undergo a few modifications. For instance, where the external zone of the outer cortex has peeled off by disorganization, the cortex of the rootlet is reduced to the few layers of the inner dark zone. Such a rootlet has been noted and figured by Williamson (17, Pl. LIII, Fig. 16) as a young *S. ficoides* rootlet. Pl. I, Fig. 4 shows a rootlet in the same state of preservation. When a rootlet has just escaped from the main axis its outer cortex is thicker than in the normal type. The outer zone of its outer cortex is five or eight layers in thickness, and the rootlet looks much more massive than the normal ones. Such a rootlet is shown in Pl. VII, Fig. 52.

A curious fact which must be pointed out is the complete absence of a glandular strand in the cortex of the normal rootlet. When (pp. 44 and 45) the course of the rootlet bundle through the outer tissue of the main axis was studied, it was pointed out that the two zones of the rootlet's cortex show a continuity of structure with the zones II and III of the main axis. The parenchymatous cells of the inner dark zone of the cortex of the rootlet are much more like the dark small-celled group found in zone II of the main axis cortex just opposite the base of the rootlet, but no secretory

strand surrounded by elongated parenchymatous cells occurs. If numerous rootlets had not been found associated with the main axis of *S. bacupensis* (Pl. I, Fig. 6), identifying them with certainty, the peculiar structure of a new rootlet species *S. arachnoidea* created by Dr. Koopmans would probably have been described as belonging to *S. bacupensis*. That rootlet possesses in its cortex very conspicuous dark groups regularly distributed, connected to one another by parenchymatous cells which are tangentially elongated. The short description and the published figure repeated (Pl. VII, Fig. 53) correspond reasonably with what has been said about the glandular strand of the outer tissue in *S. bacupensis*. Yet the author considers the dark groups of *S. arachnoidea* as sclerotic nest. This is absolutely not the case in *S. bacupensis*. Such rootlets being present in Belgian coal-balls (4, p. 18, Pl. VIII, Fig. 72, Pl. XII, Fig. 92), special attention will be drawn to them in a later paper.¹ But the analogy of structure between *S. arachnoidea*, Koop., and the outer tissues of *S. bacupensis*, Scott et Lang, is, I think, interesting to note. That example proves that, however striking a similarity of structure may be, it is never a sufficient argument to establish a relationship between two plants.

SUMMARY AND CONCLUSIONS.

The morphological and histological features which have been described show complete justification for considering *S. bacupensis* as a new species. The most striking features of the species are: the massive primary wood of the main axis consisting of spiral and barred marking elements developed without apparent direction; the glandular zone of principal outer cortex; and the well-defined structure of the free rootlet.

The first of these characteristic features is sufficient in itself to distinguish *S. bacupensis* from all known *Stigmariæ*. For so far as primary wood is concerned, the *Stigmariæ* may be divided into three types:

- | | | |
|---|---|---|
| TYPE I. <i>Stigmaria</i> with a centripetal solid primary wood consisting of scalariform tracheides. No pith. | { | <i>S. augustodulensis</i> , Renault.
<i>S. Lohesti</i> , Leclercq (6).
<i>S. dubia</i> , Scott (9). |
| TYPE II. <i>Stigmaria</i> with a well-defined ring of centripetal wood lining the pith. | { | <i>S. brardi</i> , Renault, primary xylem formed by strand of crescentic transverse section.
<i>S. weissiana</i> , Leclercq, primary xylem consisting of two or three continuous layers. |

¹ The author commits an error when he says (p. 18) that these rootlets are not present in any of the slides he has seen from Belgium.

TYPE III. *Stigmaria* with centri-fugal primary wood: the centre of the axis seems to be hollow. $\left\{ \begin{array}{l} S. ficoides, \text{ Sternb.} \\ S. radiculosa, \text{ Hick.} \\ S. petticurensis, \text{ Scott (9).} \end{array} \right.$

What place shall we confer to *S. bacupensis*? It appears that it cannot be classed in any of the above-mentioned types, for the anatomy of *S. bacupensis* seems to represent an intermediate structure between *Stigmariae* with centripetal or centrifugal primary wood.

S. bacupensis new type would then be placed between the second and third type already described:

Stigmaria with solid primary wood consisting of spiral and barred thickening elements, developed in no apparent direction. No pith. $\left\{ \begin{array}{l} S. bacupensis, \text{ Scott et Lang.} \end{array} \right.$

If *S. bacupensis* possesses a solid primary wood like *S. augustodunensis*, Ren., and *S. Lohesti*, Lecl., its secondary wood breaking out in numerous distinct wedges, the monarch structure and somewhat triangular form of the bundles of the rootlet and the course of the former, present, as has been said above, a similarity in structure to *Stigmariae* with centrifugal primary wood like *S. ficoides*.

Generally *Stigmariae* with solid primary wood developed centripetally have a compact secondary wood, very few appendages, and more or less numerous mesarch rootlet bundle. Such anatomical changes have not been introduced in *S. bacupensis* structure. I am inclined to consider the small spiral and barred elements of its primary wood as a protoxylem enlarged somewhat excessively. That hypothesis would explain the absence firstly, of direction in the primary wood development, and secondly, of the features peculiar to *Stigmariae* with a centripetal solid primary wood mentioned above.

The above classification established on a single feature has of course no specific value, but has the merit of showing that among all the *Stigmariae* known, specimens with centripetal primary wood are more numerous than specimens with centrifugal primary wood. *S. ficoides*, which is generally the very commonest of all fossils in coal-balls, has consequently been investigated first. In all handbooks the knowledge of the anatomical structure of the *Stigmariae* is based on the description of *S. ficoides*, which, however, might easily not represent the commonest structure of the *Stigmariae*.

It is advisable now to sum up the whole features of *S. bacupensis*, Scott et Lang. The dimensions of the whole axis, stele, and bark vary according to the age of the specimens. The vascular cylinder consists of a solid primary xylem made up of small spiral annular and barred marking

elements of various size, developed in no apparent direction, and of a secondary xylem broken down in numerous bundles by numerous medullary rays. The inner margin of the bundle is loose. No pith is present in the centre of the stele. Inner and middle cortex are ill preserved. The outer tissues include four zones: first, a centripetal periderm of an irregular type made up of normal prosenchyma mixed with chambered-cells; secondly, a particular glandular zone which consists of schizogenous isolated sacs of various length and width ending blindly, and filled largely with remains of cell-walls and waste products (these glandular strands are generally connected by a wide-celled parenchyma); thirdly, a parenchymatous tissue made up of moderate-sized cells which show power of division; fourthly, a delicate small-celled tissue generally broken down. A particular feature of the main axis must still be added: vascular cylinder branched off frequently. It is not uncommon to find two or more steles in a single preparation.

In relation to the main axis the monarch bundle of the rootlet shows a structure and course similar to *S. ficoides* appendages.

The free rootlet has a typical structure. The monarch bundle is connected with the outer cortex by a definite parenchymatous bridge. In this latter there frequently occur fine vascular strands which connect the woody element of the bundle with cortical tracheides. The outer cortex is divided into two zones: an inner dark one, and an outer clear one enlarged just opposite the protoxylem point of the bundle, where a patch of cortical tracheides is located. The rootlets are of small dimension, their size ranging from 1 to $1\frac{1}{2}$ mm., and they are extremely abundant. The rootlets of *S. bacupensis*, which are common in calcareous section and which cause disorganization in the structure of the plants they invade, can now be easily distinguished and identified. It will also be possible to determine with certainty the presence of *S. bacupensis* among a coal-ball flora in which the main axis is absent.

The identity of *S. bacupensis* being established, it is necessary to draw attention to the wrong determinations met with in the Scott collection.

It has been said in Introduction to this study that two preparations of Scott's collection have been chosen to figure as type specimens in this paper. Among the numerous specimens identified as *S. bacupensis* in the British Museum, some of them are doubtful. The preparations number 1085, 6 and 7 represent transverse and tangential sections through a specimen of which the following characteristic features are: centripetal primary wood ill developed, consisting of elements closely joined to one another; secondary wood well developed, few medullary rays, few appendages showing in transverse section a somewhat mesarch structure. Cortex unhappily not preserved. Both structure of primary and secondary wood show a striking difference from the same tissues in *S. bacupensis*. In these latter, as we know, the primary wood consists of very small elements developed in

no apparent direction. The secondary wood is abundantly divided in rather narrow bundles by numerous medullary rays and rootlet bundle. The monarch appendage bundle has an edged-shaped transversal section.

The structure of the specimens enclosed in the preparations number 1085, 6 and 7 is much more like *S. weissiana*, Lecl. However, as special attention has not been drawn to them, the analogy between the two plants cannot be determined here with certainty. It was, however, necessary to rectify the error of determination. The true morphological nature of Stigmarian axes is still unknown. If they belong to plants which have attained their highest development in later palaeozoic times they have proved to be more primitive in their anatomical structure than most of their recent allies. They suggest a relationship corresponding with existing Lycopods (*Lycopodium* and *Selaginella*) and *Isoetes*, but of course no strict homology with any of them may be assumed.

In the present state of our knowledge it is also fruitless to try to determine with certainty if the appendages represent modified leaves or rootlets. As Professor Lang (5, p. 104) clearly notes, until information as to the structure of the apical region of a Stigmarian axis and the mode of production of the root is known, close comparison with other plants cannot be profitably established. Yet the investigation of Professor Lang on the rootlets of *Stigmaria* demonstrating the possibility of their endogenous origin brings forward a new and weighty argument in favour of the appendages being of the nature of roots.

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EXPLANATION OF PLATES I-VII.

Illustrating Dr. S. Leclercq's paper on *Stigmara bacupensis*, Scott et Lang.

All the photographs have been taken by the author by means of 'La chambre micro-photographic' camera of Zeiss, except Pl. II, Fig. 9, in which the apparatus of Eddinger was employed, and the re-figured figures Pl. I, Figs. 3 and 5, Pl. IV, Fig. 29, and Pl. VI, Fig. 53 in which the fluorescent screens of Tall were used.

PLATE I.

Fig. 1. Stele of a young branchial axis; x_1 , primary xylem; x_2 , secondary xylem (transverse section). Prep. 950, D. H. Scott, British Museum. $\times 25$.

Fig. 2. Main axis of *S. bacupensis* cut obliquely, surrounded by numerous appendages cut transversally and longitudinally. Prep. 1773, D. H. Scott, British Museum. $\times 2$.

Fig. 3. Repeated figure of a decorticated *S. bacupensis* rootlet attributed by Williamson to a young *S. ficoides* appendage; *par.*, destroyed patch of large-celled parenchyma of outer cortex. (1881, Pl. LIII, Fig. 16.) $\times 80$.

Fig. 4. Decorticated *S. bacupensis* appendage. The outer zone of the outer cortex is peeled off; *par.*, destroyed patch of large-celled parenchyma of outer cortex. Prep. 2/1. $\times 90$.

Fig. 5. Repeated figure of an undetermined rootlet investigated by Prof. F. E. Weiss. A comparison with *S. bacupensis* type specimen rootlet establishes the identity of the two appendages (Pl. VI, Figs. 46 and 47), Weiss (1902), Pl. XXVI, Fig. 3. $\times 30$.

Fig. 6. Main axis cut transversally, surrounded by some of its numerous appendages: *st.*, stele; *c.*, cortex; *r.*, rootlet. Prep. 21/2. $\times 6\frac{1}{2}$.

Fig. 7. Transverse section of a main axis, surrounded by the outer tissues. Lettering as in Fig. 6. Prep. 624/1. $\times 2\frac{1}{2}$.

Fig. 8. Transverse section of a somewhat more developed main axis, surrounded by the outer tissues. Lettering as in Fig. 6. Prep. 678/1. $\times 2$.

Fig. 12. Tracheides of secondary wood; x_2 , joined by a row of primary wood elements of various size. Preparation made by means of the celluloid method of J. Walton. Prep. 624 1/7. $\times 140$.

PLATE II.

Fig. 9. General transverse section of the axis showing: x_1 , primary xylem; x_2 , secondary wood; *r.ba.*, rootlet base; *rb.*, rootlet bundle; *pr.*, periderm; *gs.*, glandular strand; z_3 , third zone of the outer cortex. Prep. 624/1. $\times 40$.

Fig. 10. Transverse section of the primary xylem formed by elements of various dimensions: x_1 , primary xylem; x_2 , secondary xylem. Prep. 624/1. $\times 150$.

Fig. 11. Wide element (*w.e.*) situated in the centre of the axis (see Pl. II, Fig. 10), surrounded by several smaller elements. Prep. 624/1. $\times 250$.

Fig. 12. See Plate I.

Fig. 13. Reticulate tracheides cut transversally. Prep. 624/1. $\times 270$.

PLATE III.

Fig. 14. Radial section through the solid primary xylem showing annular and barred elements of various size: *my.*, mycelium; *x₂*, secondary tracheide. Prep. 624 1/6. $\times 140$.

Fig. 15. Radial section through a stele showing (*x*) primary and (*x₂*) secondary wood in contact. Prep. 624 1/6. $\times 75$.

Fig. 16. Radial section through the primary wood showing spiral and barred elements found in its centre. Prep. 624 1/6. $\times 420$.

Fig. 17. Radial section through the primary wood showing barred elements mixed with thick bearded mycelium: *my.* Prep. 624 1/6. $\times 140$.

Fig. 18. Tangential section through secondary wood showing (*s.tr.*) scalariform tracheides; (*mr.*) medullary rays; (*rb.*) outgoing rootlet bundle. Prep. 802 1/4. $\times 14$.

Fig. 19. Radial section of an axis showing: *x.*, primary wood; *x₂*, secondary xylem; *my.*, mycelium. Prep. 624 1/6. $\times 14$.

Fig. 20. Transverse section through irregular periderm: *pr.*, files of prosenchymatous cells; *ch.*, chambered-cells; *gs.*, glandular strand; *z₃*, zone III of the outer cortex. Prep. 678 1/1. $\times 14$.

PLATE IV.

Fig. 21. A portion of periderm enlarged: *pr.*, files of prosenchyma; *ch.*, chambered-cells showing numerous secondary septa, *sp.*; *ph.*, tangentially elongated prosenchymatous cells. Prep. 678 1/1. $\times 40$.

Fig. 22. Tangential section through periderm showing the definite vertical files of chambered-cells, *ch.*, among the prosenchymatous cells, *pr.* Prep. 678 2/4. $\times 14$.

Fig. 23. Tangential section through the periderm showing the chambered-cells with their numerous daughter-cells, *dg.*, and the parallel sides and pointed end prosenchymatous cells, *pr.* Prep. 678 2/4. $\times 40$.

Fig. 24. Radial section through the outer cortex; *pr.*, prosenchymatous cells of periderm; *gs.*, glandular strand; *z₃*, parenchymatous cells of the zone III. Prep. 678 1/2. $\times 14$.

Fig. 25. Radial section through the outer cortex showing the conspicuous parenchymatous strand; *ch.* formed among the prosenchyma by numerous daughter-cells of chambered-cells. Other lettering as in Fig. 24. Prep. 678 1/2. $\times 25$.

Fig. 26. Glandular strand cut transversally filled by product of secretion. The wide-celled parenchyma, elongated tangentially and radially is well seen. Prep. 624 1/2. $\times 75$.

Fig. 27. Two glandular strands cut transversally; the right one shows a central passage; *pr.*, periderm; *z₃*, third zone of the outer cortex. Prep. 624 1/2. $\times 75$.

Fig. 28. A glandular strand in formation. Prep. 950. Scott collection, British Museum. $\times 180$.

Fig. 29. Repeated figure of Prof. W. H. Lang's paper, showing in a very young axis two glandular strands in formation: *gs.*, glandular strand. (Lang, 1923, Pl. II, Fig. 5.) $\times 50$.

PLATE V.

Fig. 30. Part of tangential section through the secondary zone of the main axis cortex to show glandular strands (*gs.*) and an outgoing appendage. Prep. 624 1/6. $\times 36$.

Fig. 31. Group of three glandular strands cut tangentially. Prep. 678 2/2. $\times 40$.

Fig. 32. One glandular strand of the above figure enlarged: *c.*, central passage filled by dark bodies; *L.*, peripheric layers of cells; *pa.*, wide-celled parenchyma tangentially elongated. Prep. 678 2/2. $\times 70$.

Fig. 33. Glandular strand cut tangentially: *c.*, central canal; *L.*, disorganized peripheric layers; *pa.*, tangentially elongated parenchyma. Prep. 678 2/2. $\times 75$.

Fig. 34. Glandular strand cut radially. *pr.*, prosenchymatous cells of periderm; *tc.*, thick wall cells. Other lettering as in Fig. 33. Prep. 678 1/2. $\times 75$.

Fig. 35. Transverse section through the third zone of the cortex. On the right group of cells showing indications of tangential divisions. Prep. 950, Scott collection, British Museum. $\times 70$.

Fig. 36. Cortex of a young axis in transverse section showing the outermost zone: z_4 . Prep. 2 1/2. $\times 20$.

Fig. 37. Radially cut axis showing a bundle appendage running horizontally through secondary xylem; x_1 , crushing primary xylem; x_2 , secondary tracheides; r , rootlet bundle. Prep. 678 1/2. $\times 40$.

PLATE VI.

Fig. 38. Part of radial section to show rootlet bundle incurved bluntly when leaving off the secondary wood, x_2 ; mc , disorganized middle cortex. Preparation obtained by means of new celluloid method of J. Walton. Prep. 624 1/5. $\times 36$.

Fig. 39. Part of radial section to show the very oblique course taken by rootlet bundle through middle cortex region: x_1 , primary wood; x_2 , secondary wood. Prep. 624 1/6. $\times 14$.

Fig. 40 and 40 bis. Part of tangential section through wood, showing an outgoing rootlet bundle: tr , tracheide scalariform; mr , medullary rays; px , protoxylem of rootlet bundle. Prep. 802 1. $\times 36$. Prep. 802 1/4. $\times 40$.

Fig. 41. Transverse section of a rootlet bundle (rb); passing through periderm (pr). Prep. 678 2/4. $\times 70$.

Fig. 42. Transverse section of a rootlet bundle (rb) passing through the second zone of the main axis cortex. The first zone of the rootlet cortex is formed. Prep. 624 1/6. $\times 36$.

Fig. 43. Transverse section of a rootlet bundle running through the third zone of the main axis cortex. The second zone of the rootlet bundle is formed. On the right of the figure, some cells show indications of tangential divisions. Prep. 678 2/2. $\times 36$.

Fig. 44. Part of transverse section to show two bases of rootlet: rt , rootlet bundle; ic , inner cortex; mc , remains of middle cortex; oc , outer cortex; z_4 , outermost zone of the main axis cortex crushed between the two rootlets. The continuity of the second and third zones of the outer cortex of the main axis with the two zones of the rootlet cortex is well seen. Prep. 624 1/2. $\times 25$.

Fig. 45. Transverse section of rootlet bundle passing through the middle cortex region of the main axis: px , protoxylem; tr , tracheides scalariform; ic , remains of inner cortex. Prep. 624 1. $\times 70$.

Fig. 46. Transverse section of a free rootlet; rb , rootlet bundle; ic , inner cortex; w , remains of middle cortex cells-wall; br , bridge of parenchyma; vs , fine vascular strand; iz , inner dark zone of the outer cortex; oz , outer zone of the outer cortex considerably enlarged just opposite the protoxylem point of the bundle. Prep. 2 1/2. $\times 40$.

Fig. 47. Transverse section of a group of rootlets. On the right of the figure a rootlet with remains of middle cortex. Prep. 2 1/2. $\times 20$.

Fig. 48. Rootlet with (mc) middle cortex preserved. Prep. 2 1/2. $\times 45$.

PLATE VII.

Fig. 49. Oblique section through a *S. bacupensis* rootlet showing a delicate strand of tracheide (vs) starting from the rootlet bundle (rb). Prep. 624 2/3. $\times 25$.

Fig. 49 bis. Part of the above section magnified; vs , fine vascular strand; ic , inner cortex; rb , portion of rootlet bundle. Prep. 624 2/3. $\times 100$.

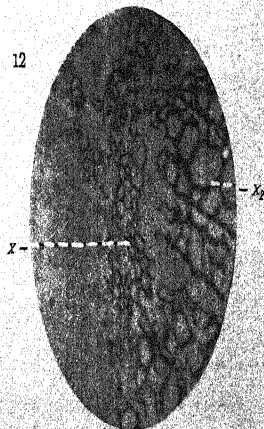
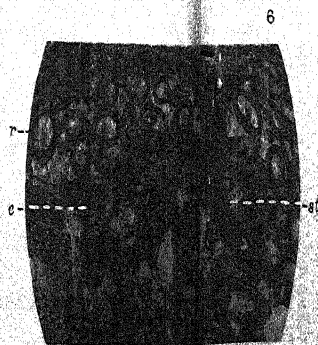
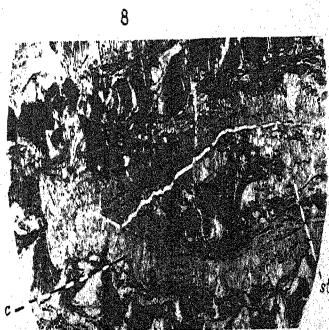
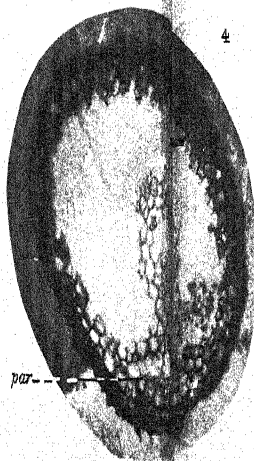
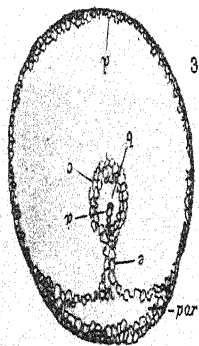
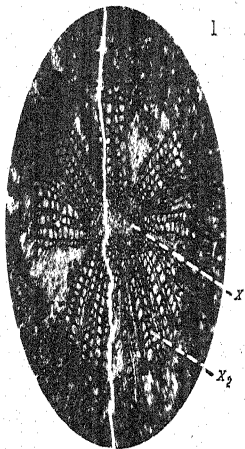
Fig. 50. Radial section passing just through the large-celled portion, ic , of the outermost zone of the outer cortex of a rootlet: iz , inner dark zone; oz , outer light zone; rb , rootlet bundle. Prep. 624 2. $\times 15$.

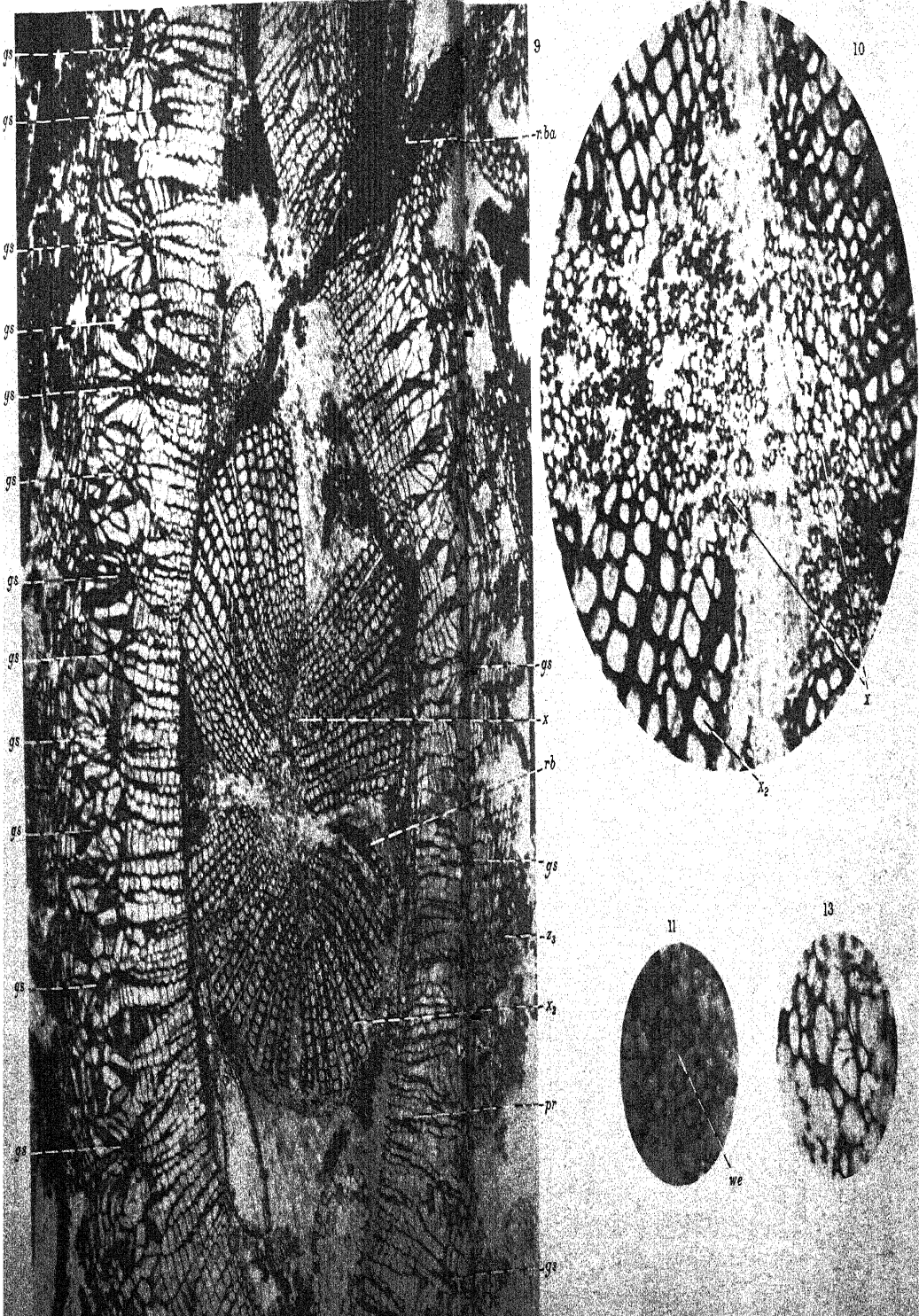
Fig. 51. Part of the above radial section magnified showing wide spirally marked cortical tracheides, ct . Other lettering as in Fig. 50. Prep. 624 2. $\times 100$.

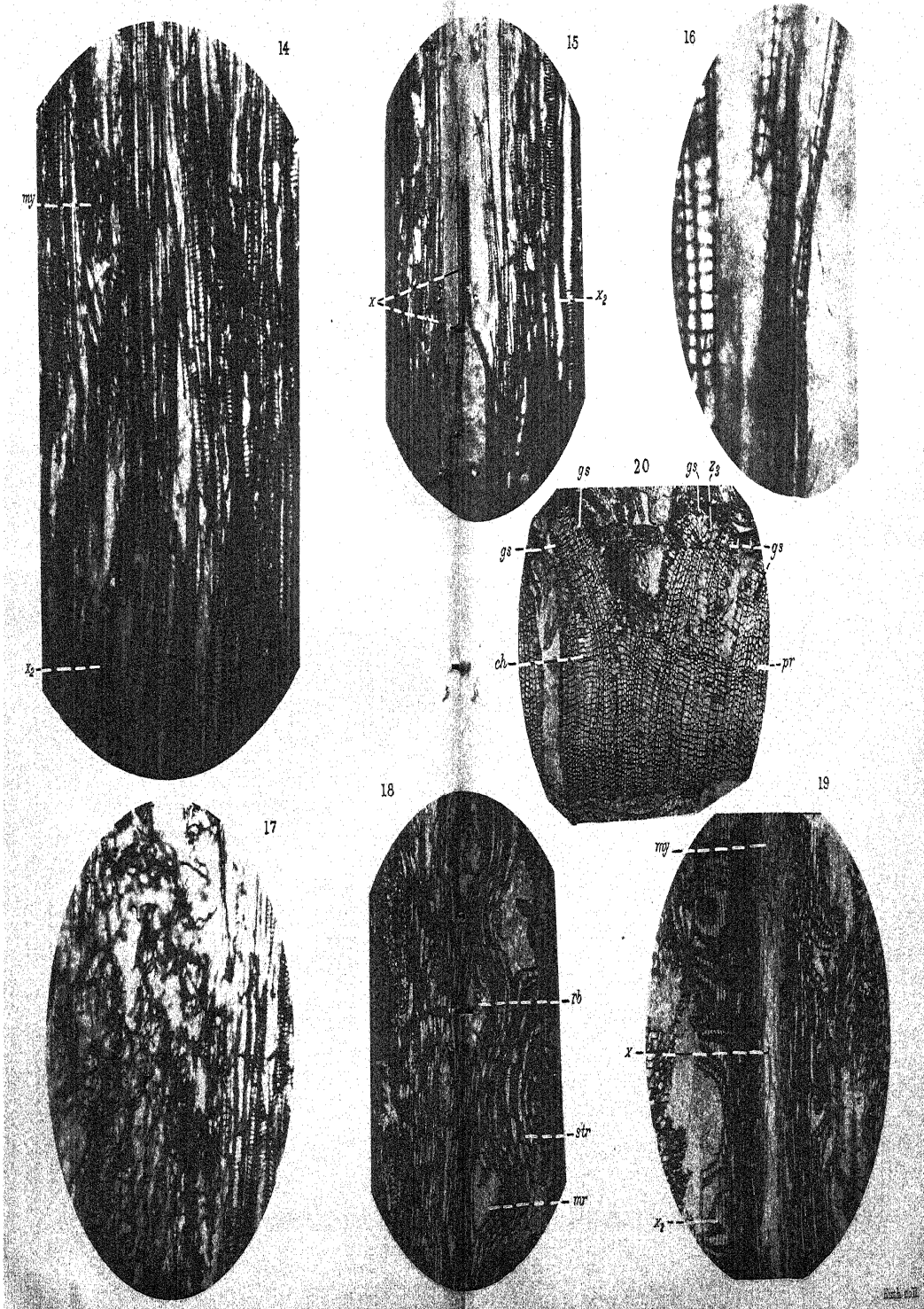
Fig. 52. Transverse section of a rootlet just escaped from the main axis: the outer cortex is thicker than in normal free rootlet. Prep. 1773, Scott collection, British Museum. $\times 36$.

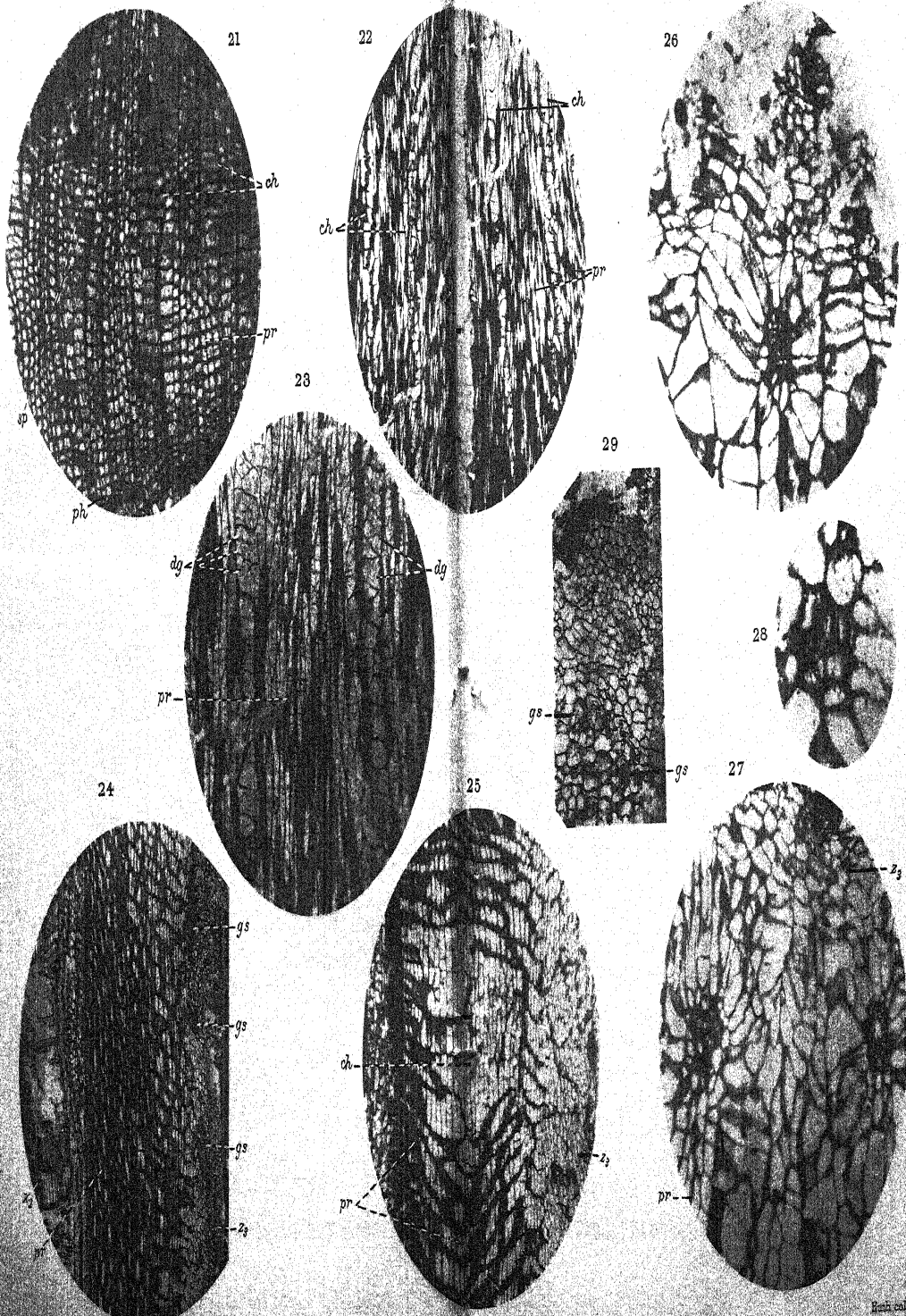
Fig. 53. Repeated figure of Koopmans new species of Stigmarian rootlet: *Stigmaria arachnoidea*. (Koopmans R. G. (1928), p. 18, Pl. XII, Fig. 92.) $\times 25$.

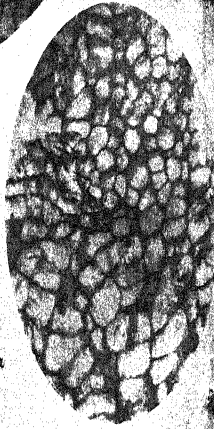
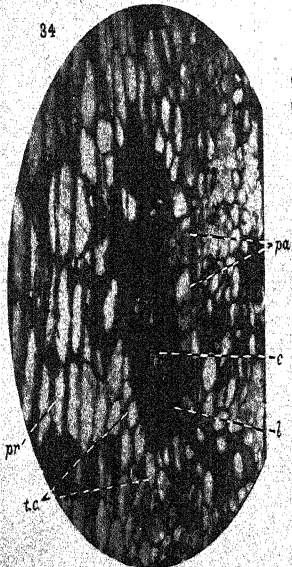
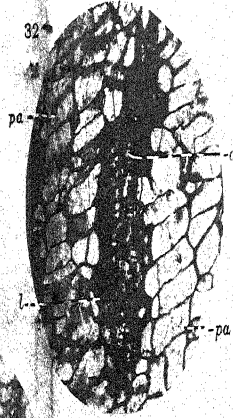
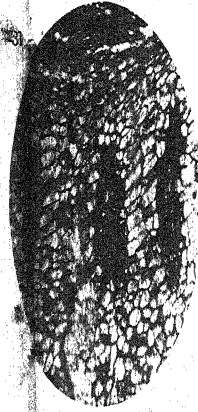
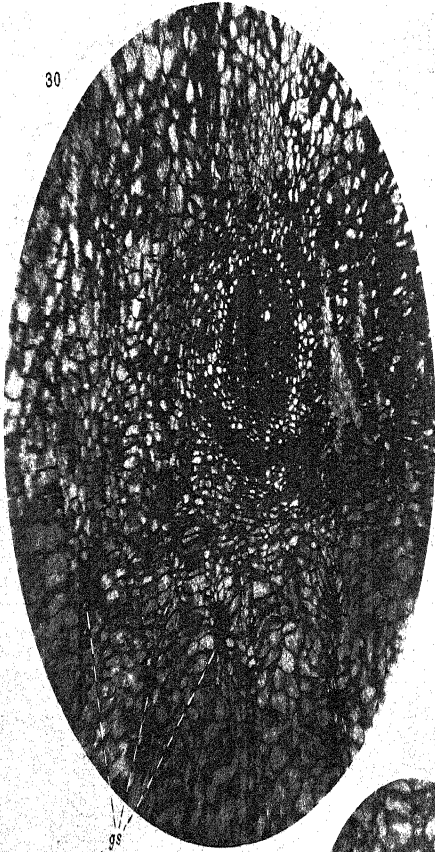
Fig. 54. Part of transverse section to show three outgoing rootlets; remains of wide cells of rootlet middle cortex, mc ; projections of outermost zone of main axis outer cortex, z_4 . Prep. 624 1/2. $\times 25$.





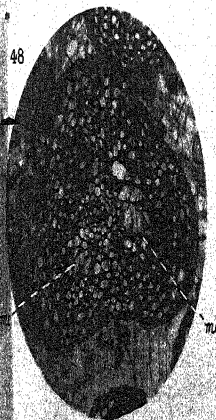
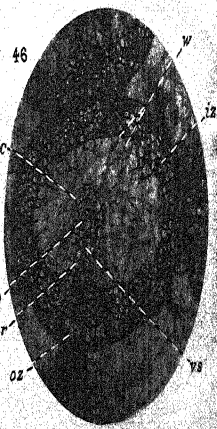
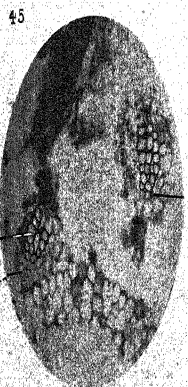
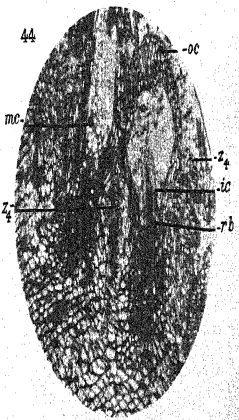
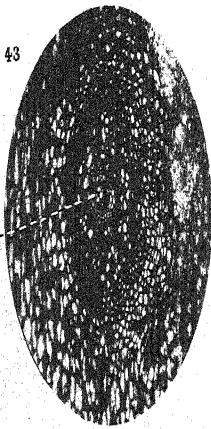
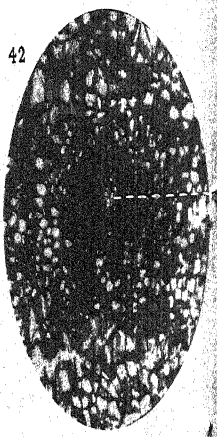
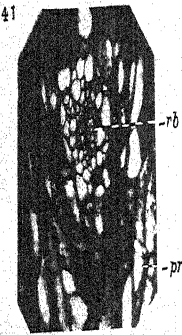
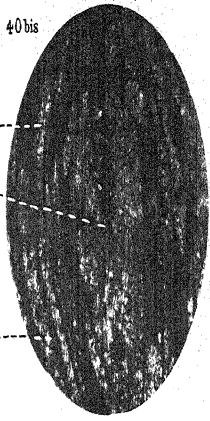
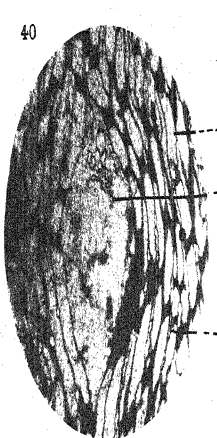


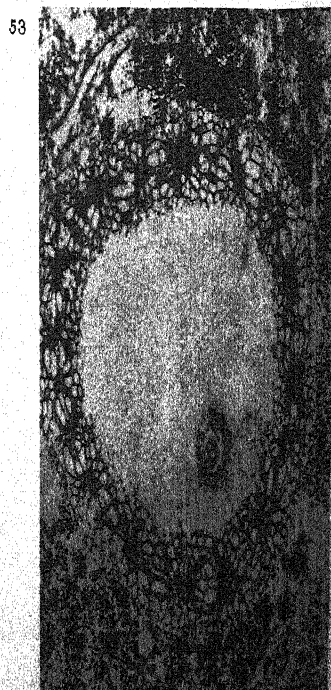
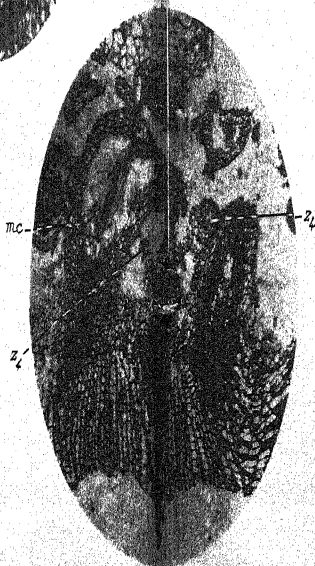
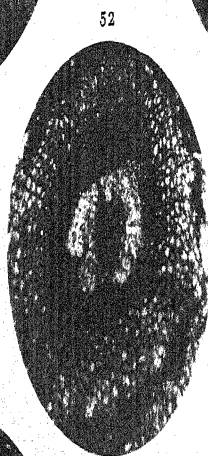
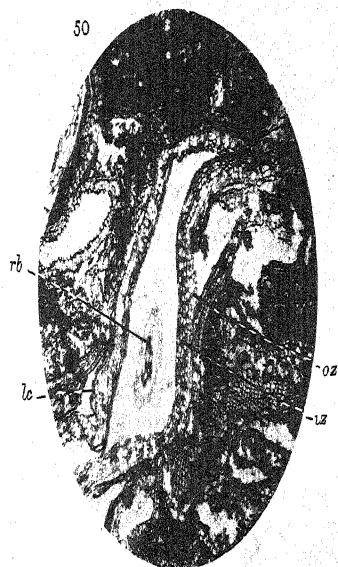
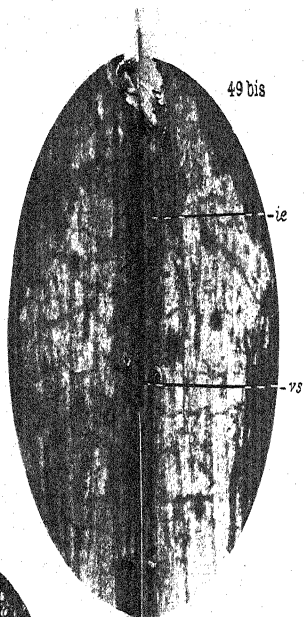
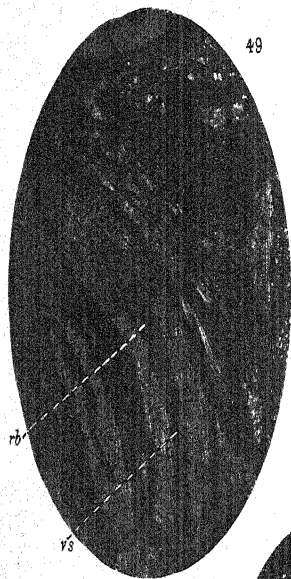




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Studies in the Root and Shoot Growth of the Strawberry.

V. The Origin, Development, and Function of the Roots of the Cultivated Strawberry (*Fragaria Virginiana* X *Chiloensis*).

BY

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With Plates VIII-X and fourteen Figures in the Text.

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I. INTRODUCTION.

IN the course of a series of investigations on the Strawberry, in progress at this station, it became increasingly evident that a much more precise knowledge of the root system of the normal plant was essential to the correct interpretation of results obtained. With this object in view a series of studies (1, 2, 9, and 10) was commenced by the pomologist and the writer in the early autumn of 1924. The normal development of the root system of the Strawberry has been closely followed through the greater part of three seasons from the date of planting. The results of this work have been published elsewhere (9 and 10), but the main features will be recapitulated in the present communication. Much of the work described herein has been carried out concurrently with these investigations, but the results obtained have been supplemented with further material.

In horticultural practice the Strawberry is propagated vegetatively almost entirely, and it is only in the development of new varieties that plants are grown from seed. Young 'runners' are planted usually in the late summer and early autumn, and bear a crop of fruit in the early summer of the following year. After three seasons the profitable life of the plant to the commercial Strawberry-grower is usually over, and it is this period which has been covered by the results presented.

The roots of a Strawberry plant derived from a 'runner' are entirely adventitious, and well-defined phases mark their formation and subsequent development. Adventitious roots first make their appearance as a young daughter-plant develops from the tip of a stolon arising from the axil of a leaf on the parent plant. These roots develop rapidly and branch freely, so that within a space of approximately one month, the stolon connecting daughter and parent may be severed, and the young 'runner' transplanted to the new bed. The rhizome or 'crown' of the young plant grows rapidly, and further adventitious roots arise from the base of the crown, usually in small groups on either side of the bases of the lower leaves. These roots grow very rapidly in the period between transplanting and dormancy, and often attain a length of 20 cm. They are usually white in colour and unbranched, or bearing only a very few lateral roots, in marked contrast to the original 'runner' roots which are yellow-brown in colour when the dormant season is reached.

From the middle of December until February, little change in the root system is observable. During the following month there is a gradual colour change, from yellow-brown to brown in the case of the branched 'runner' roots, and from white to yellow-brown in the older portions of the autumn-formed roots. From the latter, numerous branch roots to the fourth order arise during the spring. It thus appears that the vigorous roots, developed in the autumn, constitute the framework on which is built up the extensive fibrous root system necessary to support the plant through the flowering and cropping period. Very few new adventitious roots arise directly from the crown during this period. During the period of flowering and fruiting, there is a pronounced browning and decortication of the older portions of the main adventitious roots. This is presumably due to the formation of a deep-seated cork layer, for these blackened roots invariably show a healthy central core, and are well furnished with vigorous, living, lateral roots.

This condition of the root system obtains until July when new adventitious roots begin to appear. These roots have their origin near the bases of the leaves, and at a level on the rhizome (or crown), some 2-3 cm. above that of the older roots. Subsequent development of these new roots is very rapid, so that they constitute approximately one half of the total root system of the plant by the time the dormant season is reached. In general appearance the roots thus formed, especially those arising in August and September, resemble very closely those produced in the previous autumn.

With minor deviations, attributable to seasonal variations, this sequence of root development has been fully confirmed by continued observation over nearly three seasons, both on the original plants and on further series of similar plants. It has also been shown that differences of soil and locality do not materially affect this periodic sequence. Stages in the development

of the root system of a normal plant are illustrated in Pl. VIII, Figs. 15, 16, and 17.

So far as the investigation has been carried no indication of normal death or degeneration of the older main roots has been observed with the ageing of the plant. The older roots continue to develop in thickness, so that examination of a three year old plant shows the original 'runner' roots and the strong main roots representative of three periods of vigorous root formation.

The present communication deals with the histological structure of the root, through the successive stages of development, and the physiological significance of the specialized structures is discussed.

II. MATERIAL AND METHODS.

Young stolons, in various stages of development, from a few centimetres in length up to the establishment of the first 'runner', were collected in June and suitably preserved. This material was used in tracing the origin and early development of the first daughter-plant, with special reference to the origin of the first adventitious roots.

At intervals of from three to six weeks throughout the period of the study, samples of the various roots have been collected and preserved. From the examination of these samples it has been possible to follow the development of the normal adventitious root through each stage to maturity. And further, since a vigorous formation of new roots of this type occurs each year, it has been possible to repeat the examination during three consecutive seasons. This material has also been examined micro-chemically.

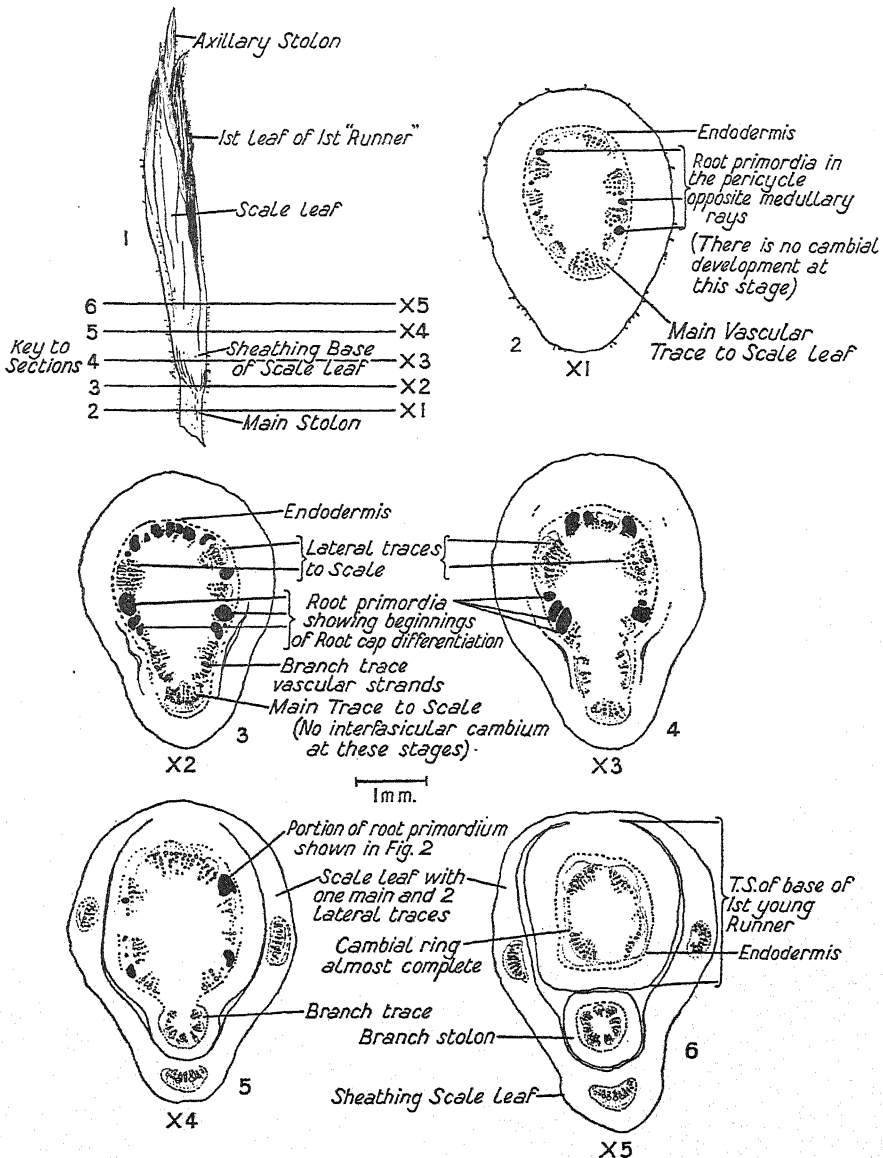
Where necessary the samples collected from the field have been supplemented with material from pot plants grown under controlled conditions.

An automatic freezing microtome by Leitz has been used throughout the work, and has proved eminently satisfactory.

III. THE DEVELOPMENT OF THE 'RUNNER'.

The growing point of a normal Strawberry stolon is enveloped in a sheathing bract, the base of which completely encircles the stolon axis, and which may show the rudiments of the three leaflets at its apex. At a slightly later stage the young leaves of the first 'runner' emerge from the bract, closely followed in many cases by a young stolon with its growing point also enclosed in a sheathing bract. At first sight it appears that the first leaves arise from a lateral bud on the stolon, which eventually develops adventitious roots and forms a daughter-plant or 'runner'. Anatomical examination of the young stolon and 'runner' at this stage shows quite con-

clusively that this is not so, the first 'runner' is developed from the growing point of the stolon, and the apparent extension of the original stolon is

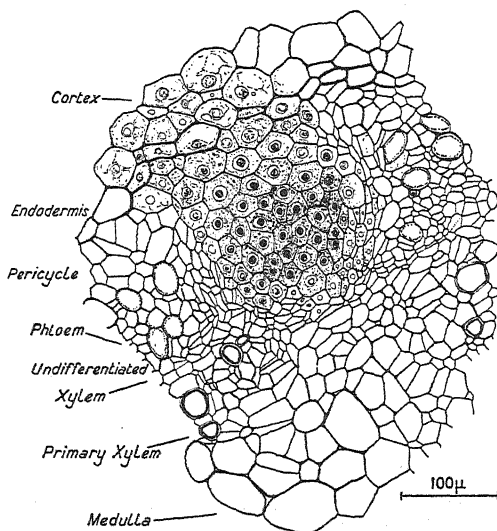


TEXT-FIGS. 1-6. Figs. 2-6. Camera lucida drawings from serial sections of a young stolon, seen in Fig. 1, showing origin of 'runner crown' adventitious roots and lateral stolon.

carried on by a lateral bud arising in the axil of the bract below the original growing point. Branching is therefore typically sympodial, and there is no

essential morphological difference between the first 'runner' on a stolon and a branch 'crown' on the parent plant.

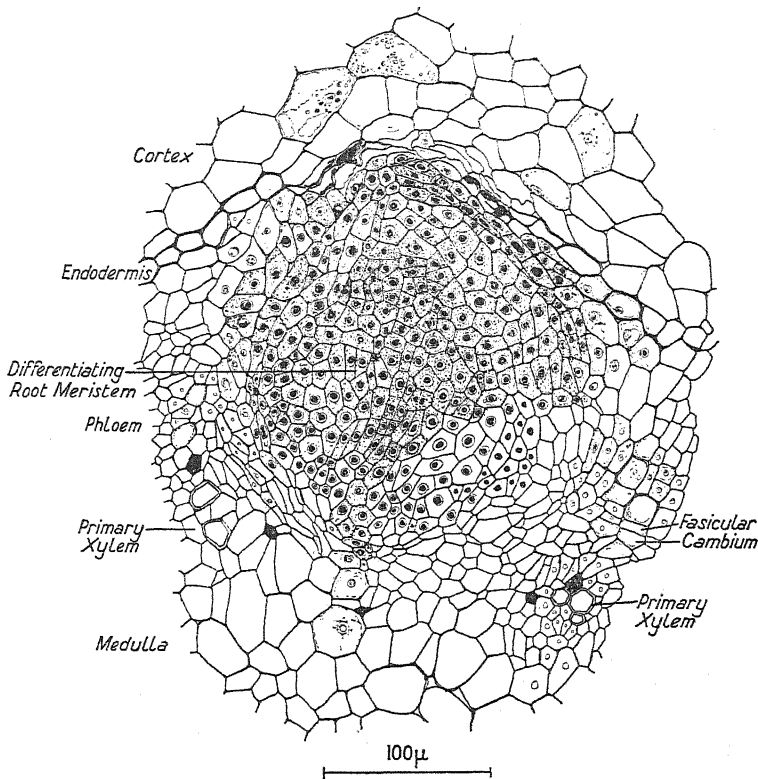
An early stage in the development of stolon and 'runner' is sketched in Text-fig. 1. Serial sections were made of similar specimens from a point below the fusion of the sheathing bract and stolon (2, *X* 1) to a point above



TEXT-FIG. 7. Young adventitious root initial seen in transverse section from the section shown in text-fig. 3.

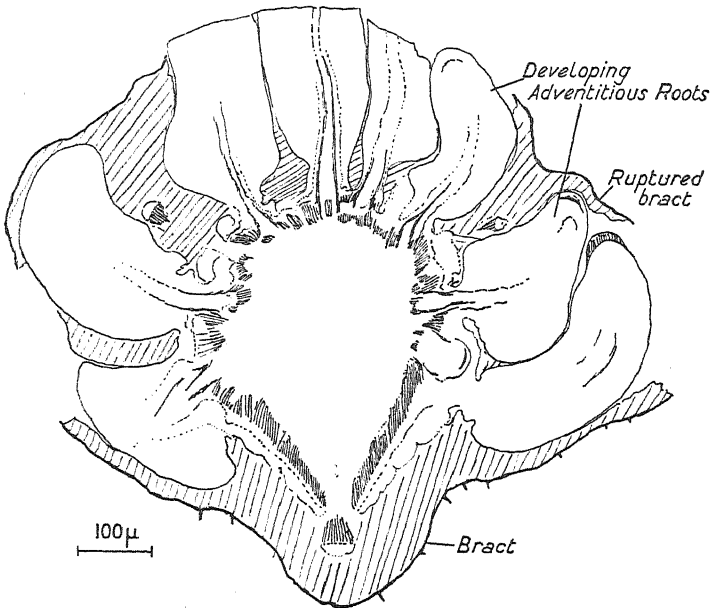
the departure of the branch stele to the lateral stolon, and the closure of the gap in the main vascular system (6, *X* 5). Through this region the vascular system, which forms a compact cylinder in the stolon, opens somewhat abruptly to form a wide cone towards a broad growing point. At the same time the main vascular trace followed by the right and left lateral traces to the scale leaf depart, in this order, from the stele (Text-figs. 2, 3, 4, and 5). The vascular cylinder to the branch stolon, which is fused at the base to the main leaf-trace, is rapidly completed, and the foliar and branch gaps in the main stele are quickly closed (Text-figs. 5 and 6). In the region immediately above the departure of the leaf-traces, the central stele shows very little differentiation of vascular tissues. A large-celled medulla is bounded by a much broken ring of xylem, in which only the primary elements are developed, and a wide pericycle limited by a clearly defined endodermis surrounds a similarly undifferentiated phloem. At this stage there is no indication of vascular cambial development except in the actual leaf-trace bundles. Occupying the positions between the leaf-trace bundles root primordia (Text-fig. 7) can be clearly distinguished. In preparations stained with haematoxylin or gentian violet the small groups of meristematic cells of root primordia are shown. These are illustrated diagrammatically

in Text-figs. 2, 3, 4, and 5. It is clear that these adventitious roots have their origin, not from a secondary meristem, but from meristematic tissue

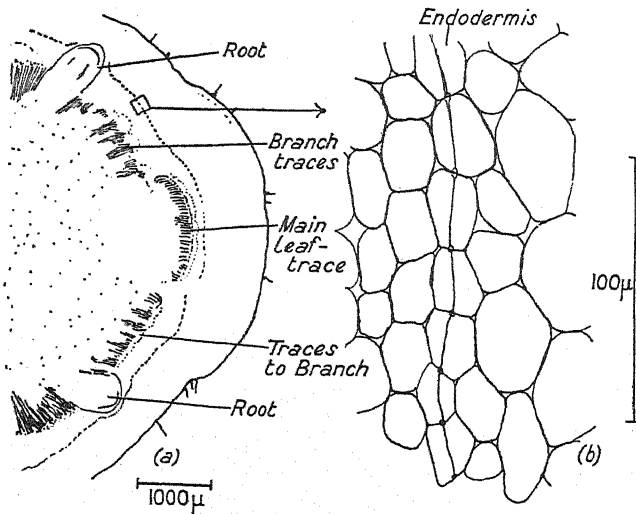


TEXT-FIG. 8. Later stage in the development of adventitious root initial. Note first indications of fascicular cambium may be observed.

which has probably retained its meristematic condition from its origin at the growing point. The detailed structure of a typical root primordium is shown in Pl. I, Fig. 1. With the increase in age of the 'runner', the roots develop rapidly, penetrating the endodermis and cortex (Text-figs. 8 and 9), and turning downwards into the soil in two fairly well-defined groups, one on either side of the connecting stolon. These roots collectively form the 'runner' root system, and it is the usual practice, when some three or four weeks have been allowed for their development, to sever the stolon and transplant the young 'runner'. As new leaves arise further adventitious roots have their origin close to the edges of the leaf-gaps, in a manner essentially the same as that described for the first-formed roots (Text-fig. 10). Both in the young 'runner' and in the rhizome a primary endodermis, the cells of which frequently contain starch in the early stages, can be distinguished, but its continuity is much interrupted.



TEXT-FIG. 9. Semi-diagrammatic transverse section of young 'runner' showing relation of adventitious roots to the young rhizome of the developing 'runner'.



TEXT-FIG. 10. (a) Showing the origin of adventitious roots in the crown of an old plant; (b) detail of endodermis structure in (a).

IV. HISTOLOGY AND PHYSIOLOGY OF ROOT DEVELOPMENT.

The adventitious roots develop rapidly, often attaining a length of 20 cm. in a few weeks. They are for the most part unbranched, and almost white in colour. The majority retain the primary structure throughout this period of rapid development, and even the differentiation of vascular tissues is notably slow. In one or two specimens examined there was no sign of xylem differentiation in the young stele from the apex to some 2-4 cm. behind the growing point. The beginnings of secondary development may be observed in some of the older, first-formed 'runner' roots, but the greater proportion enter the dormant period in mid-December in the primary condition. In transverse section the root exhibits normal dicotyledonous characters. Protoxylem strands vary from five to eight in number, the pentarch formation being the most frequent. The medulla varies in quantity with the size of the root, and scattered lignified cells are of common occurrence in the pith. The pericycle is three to four cells deep, and invariably contains starch. A well-marked endodermis, which retains its primary character for a considerable distance behind the growing point, is a characteristic feature of the root at this stage. In relation to the vascular tissues the cortex is extremely bulky, and the cells of the inner cortex show a tendency towards radial formation.

During August, when rapid root growth is proceeding, there is little accumulation of starch or protein material in the tissues. Though small quantities of starch may be observed at any time in the pericycle, it is not until October and November that reserves of this material are observed in quantity. Storage begins first in the parenchymatous tissues of the stele and, later, general accumulation is evidenced in the cortex. In November and early December the accumulation is rapid, so that by the time the dormant season is reached it seems that every available parenchymatous cell is packed with starch to full capacity. Tests for fatty materials with Sudan III and Osmic acid show that these are present in considerable quantity in the cells of the pericycle. Sections of both fresh and fixed material show the presence of minute globules of fatty substance in association with starch grains in the cells of this region. Sections stained with Sudan III show that secondary suberization of the endodermis is complete, and cells of the outer one or two layers of the cortex also show pronounced suberization.

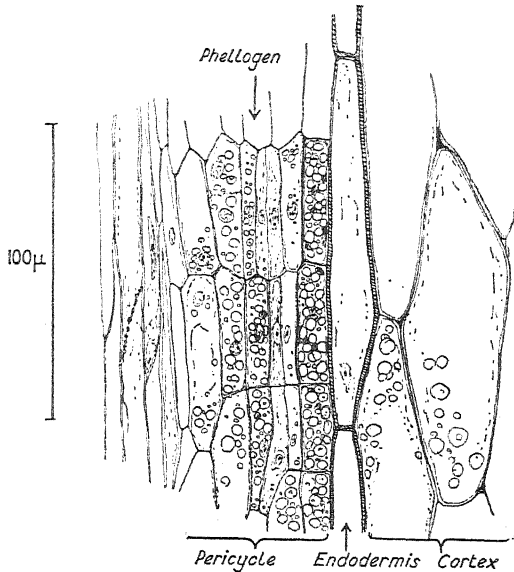
In this condition the dormant season is passed, and little histological or physiological change is observed until the latter half of January.

The first indications of emergence from the dormant state are physiological. Slowly at first, becoming rapid during February and early March, there is a general depletion of the large reserves of starch contained in the bulky parenchymatous tissues of the root. Suberization of the outer cells

of the cortex becomes more marked, and in this connexion it is of interest to note that many of the cells which became suberized in the late autumn, after starch had accumulated within them, retain this stored material when cells deeper in the cortex are exhausted. These physiological changes are closely followed by a general renewal of the meristematic activity. The beginnings of numerous lateral root initials are observed in the pericycle opposite to the primary medullary rays, and the normal vascular cambium begins to differentiate. At the same time a second cambium cycle is observed in process of differentiation in the outer one or two rows of the pericycle cells. The first indications of this meristem differentiation are observed in the pericycle tissue opposite the protoxylem groups (Pl. VIII, Fig. 2, and Text-fig. 11). This second cambium constitutes the phellogen, and its subsequent activity gives rise to a highly specialized polyderm. Through the period of rapid spring growth, when foliage and flowers are in process of development, marked changes are observed in the external appearance of the root system as a whole, and in the internal structure of individual roots. There is a prolific development of fine lateral roots of the second, third, and higher orders which are well furnished with root hairs, and the colour of the main roots changes rapidly from yellow-brown to dark brown, and finally to black. The lateral roots vary from pentarch to diarch in primary structure, and normal medulla is absent as a rule. Occasionally the secondary roots, at their union with the main root show a trace of true medulla in which lignified, pitted cells occur, but the stele of the lateral roots generally contains a central strand composed entirely of true xylem elements. The formation of a secondary vascular cambium takes place rapidly, but with very few exceptions, no trace of the phellogen derived from the pericycle was observed in the lateral roots. Subsequent observations show that the fine lateral roots have a comparatively short life. Towards the end of the fruiting period death and decay of ultimate lateral roots of the higher orders is observed. Frequently it has been observed that the whole of a fibrous root system developed on a lateral root of the first order, has decayed to the point of union with the main adventitious root. In the subsequent rapid secondary growth of the main root, the scar of the union with the decayed lateral root may be completely covered by new tissue, but occasionally new lateral roots arise in close proximity to the original secondary root as stated above. New lateral roots are for the most part developed on new adventitious roots formed in the autumn, and the period of their most rapid development occurs in the spring of the following season.

Secondary developments in the main roots are rapid; the secondary increase in vascular tissues presents no features of especial interest, but the phellogen gives rise to a highly specialized tissue, the development of which it is proposed to describe in detail (Text-fig. 11).

With the completion of the ring of phellogen cells in the pericycle tangential division rapidly takes place. The first cycle of cells cut off shows early indications of general suberization. At irregular intervals in the cycle, single cells, or pairs of cells, do not become suberized, and these often



TEXT-FIG. 11. Origin of phellogen in a young root of the strawberry seen in longitudinal section.

show radial division in process. Cycles of cells arising from the subsequent three or four tangential divisions of the phellogen are not suberized, but retain their thin cellulose walls, and often contain small quantities of starch. The cells of the next cycle again become suberized, and there is a similar occurrence of unsuberized cells, showing radial division, at intervals in the cycle. A feature which may be of considerable importance is observed in this connexion; the unsuberized cells of the second cycle occur on alternating radii with the unsuberized groups of the first cycle, to coin a term, the unsuberized regions of the two cycles do not register with one another. Longitudinal sections show that the unsuberized cells occur in small areas, and their relation to the surrounding cork cells may be compared with that of medullary ray cells to the wood elements in a tangential section. The development of this tissue appears to be almost simultaneous in the main roots, and in the lower portion of the rhizome ('crown') at the junctions of the roots and shoot. In effect, the whole of the vascular system of the portion of the plant in which secondary growth has occurred, is bounded on the outer side by an unbroken zone of muriform tissue derived from a pericyclic phellogen.

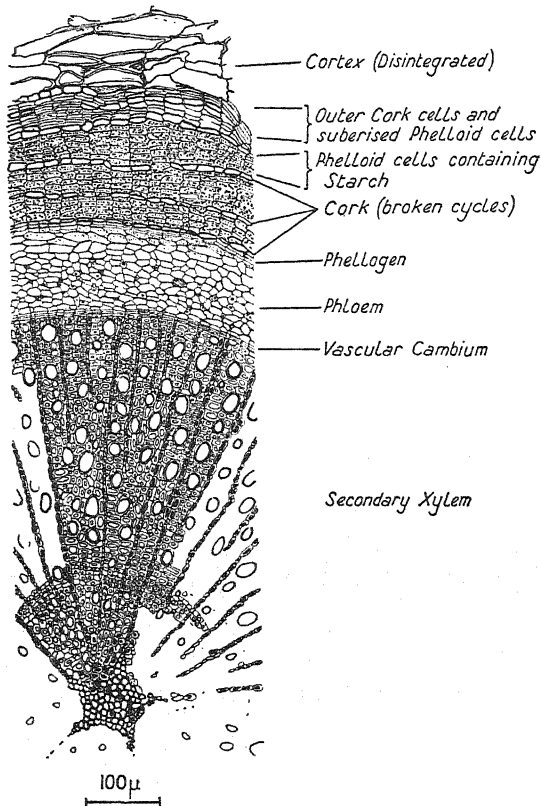
Active division of the phellogen is observed during May and June, during the normal flowering and fruiting period, and is accompanied by the external colour change of the roots previously mentioned. During this period the cortex decays, and with the development of the phellogen, though the cortical cells may still retain their form, there is no trace remaining of the large stores of starch observed in February. Disintegration of the cortex follows rapidly on the formation of the first cycle of suberized cells within the pericycle, immediately outside which the ruptured fragments of the original endodermis can be distinguished in later stages. After the flowering period towards the end of May, and in early June, accumulation of starch commences in the medullary tissue of the root, and in the unsuberized cells of the polyderm (11). This period is characterized externally by a marked retardation of growth, few new leaves are developed, and examination of the root system shows that many fine lateral roots are no longer functioning. In these respects the plants in process of fruiting differ markedly from others, which are prevented from doing so by removal of the young inflorescences in early May (9). Deblossomed plants continue to grow vigorously, retain their lateral roots in a functional condition for a longer period, and develop new leaves and young stolons at an earlier date than plants bearing fruit. The accumulation of starch in the rhizomes and roots of these plants also occurs during late June and early July, though not to such a marked extent as in the case of plants which have borne a crop of fruit. In the latter half of June, and during July, new leaves and young stolons appear on normal plants which have borne fruit. The accumulation of starch is very marked at this period, the polyderm of the older roots containing large quantities, and the plant enters on a second phase of vigorous vegetative growth. Towards the end of July new adventitious roots arise from the rhizome. These have their origin in small groups to the right and left of the bases of the leaves developed in the spring, which now show signs of approaching death. Growth is very vigorous, and under favourable cultural conditions the young roots often penetrate the sheathing, living bases of the older leaves. In their subsequent development these roots follow essentially the same course as the roots arising from the young 'runner' plant.

Up to the time of the appearance of the new adventitious roots, the older roots show an increase in thickness, and progressive decline of the fibrous lateral roots is noted. In transverse sections of older roots at this stage an increase in the development of the polyderm is observed. Three or four cycles of suberized cells can be distinguished alternating with zones of regular thin-walled cells containing numerous starch grains. With the development of new adventitious roots from the rhizome, new lateral roots occasionally develop from the older main roots, and there is a marked diminution in the amount of accumulated starch deposited in the tissues

towards the end of the fruiting period, and during the early part of the second growth phase. In the subsequent period of vigorous summer and early autumn growth, there is a rapid increase in development of the root-system as a whole. The new adventitious roots increase in length and number, the older roots develop in thickness, and a few new laterals may arise near the original points of departure of previous laterals which have decayed. Carbohydrate reserves accumulate in the bulky cortical tissues of new roots, and in the specialized unsuberized cells of the polyderm of older roots. Transverse sections of both fresh and preserved roots, stained with Sudan III and mounted in glycerine, in which a small amount of iodine was dissolved, were used in the examination of the polyderm. This technique was found to give excellent differentiation of the tissues, the bright red stain absorbed by the walls of suberized cells contrasting well with the clear blue colour of the starch grains filling the cells of the unsuberized tissue zones. The oldest roots showed as many as eight zones of unsuberized cells containing starch, alternating with the single-celled discontinuous cycles of cork cells containing no starch. During the month of November there is a very marked increase in the rate of starch accumulation, both in the cortex of young roots and in the tissues of older roots, so that when the dormant season is reached in December, it seems that every available parenchymatous cell is packed with starch to full capacity. This is especially true of the medullary and polyderm tissues of the older roots. At the same time the cells of the outer zone of the polyderm tissue, which were observed to contain starch in the early stages of the post-fruiting period of growth, now show general suberization and absence of included starch. In this way a complete outer zone of true cork is produced from three to six cells thick, and a well-protected outer coat is thus presented to the soil before the winter conditions set in. The general and detailed structure of the polyderm is shown in Text-figs. 12 and 13, and in Pl. VIII, Figs. 3-6, and Pl. IX, Fig. 7.

In this condition the dormant season is passed, and there is little structural or physiological change until February. The disappearance of starch from the cortex of younger roots is again observed, and follows a precisely similar course to that observed in the first year. Starch also begins to disappear from the polyderm of the older roots. In this respect the secondary tissue of phellogenetic origin seems to bear a similar relation to the stele of an older root as the cortex to the vascular tissues of the young root developed in the previous late summer and autumn. Depletion is rapid in March and April, and at the same time there is increased suberization in the thin-walled cells of the outer zones of the polyderm now devoid of starch content. Tests with fat stains (Sudan III, osmic acid, and cotton red) show that, in addition to the deposition of fatty compounds in the cell-walls, considerable quantities of free fat are present in the outer living

cells of the polyderm, and in the cells of the pericycle immediately within the phellogen (Pl. IX, Fig. 7). During the flowering and fruiting months of May and June there is a further increase in the activity of the phellogen,

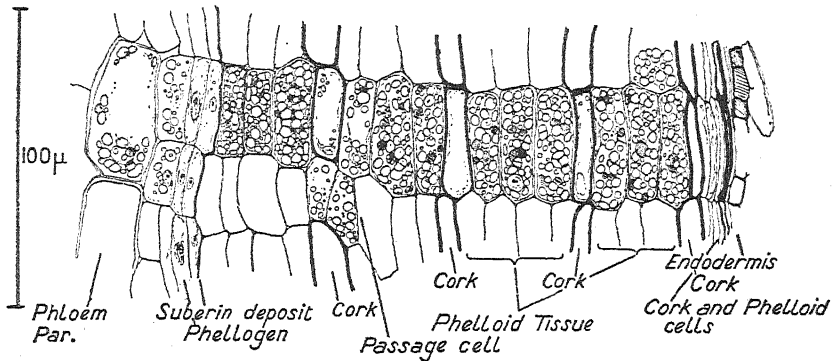


TEXT-FIG. 12. The structure of a normal root from an older plant (fourteen months).

concurrent with phellogen development, in the younger roots which follows a similar course to that described previously for the older roots in their first season. At this time the outer zones of the polyderm, which have undergone complete suberization, show signs of splitting and decay. The development of secondary tissues in all the roots is rapid during July and the later half of summer, and a further development of new adventitious roots from the younger portions of the branched rhizome occurs in a manner essentially the same as that already described. In the same way starch accumulation in the newly-formed polyderm zones is observed, and suberization of the cells of the outermost zone of living tissues takes place before the dormant season is reached. The subsequent development in the third season of the older roots, and of the new roots formed in the post-fruiting

period, does not differ in essential features from that described for the second season.

An attempt has been made to express quantitatively the relation between the polyderm tissues and the total living tissues of the root. Accurate camera lucida drawings of transverse sections of roots (in which



TEXT-FIG. 13. Detailed structure of the polyderm of the root figured in text-fig. 12.

secondary growth had commenced), made at intervals of 1 cm. throughout their length, showed on measurement a very constant relation between the total diameter of the root and the thickness of the polyderm ring. This being so, the volume relation between the polyderm and the tissues of the root as a whole, will be proportional to the surface area of the respective tissues, when viewed in transverse sections made at right angles with the main axis of the root. On this assumption the results presented in Table I have been obtained. For each period included in the Table, a large number of transverse sections of typical roots were examined, and camera lucida drawings were made at a constant magnification. Planimeter determinations of the surface area of the whole section and of the several component tissue zones were made. From the figures obtained the mean proportional areas of the several tissues were calculated. Though at a specified period there is great variation in the actual size of the roots of a definite type, the constancy of structure is clearly shown by the very small variation in the proportional relation of the tissues, that is, on the assumption explained above, the volume relations are constant. In Table I are summarized the means of twenty to thirty determinations, in each case of proportional figures (total volume = 100), expressing this volume relationship of the tissues in roots of different ages from five to twenty-five months. The general relation of the tissues is further illustrated in Text-fig. 14.

The figures presented in Table I show very clearly the marked, constant relation of the polyderm to the total volume of functional tissues of the main roots in which secondary development has taken place. If the

relatively small amount of cork included in the polyderm be discounted, the results show that approximately one-third of the volume of the root is composed of muriform, unsuberized cells derived from the pericycle phellogen.

TABLE I.

Relative Proportions by Volume of the Several Tissues of the Root of the Strawberry Plant at Different Stages of Development.

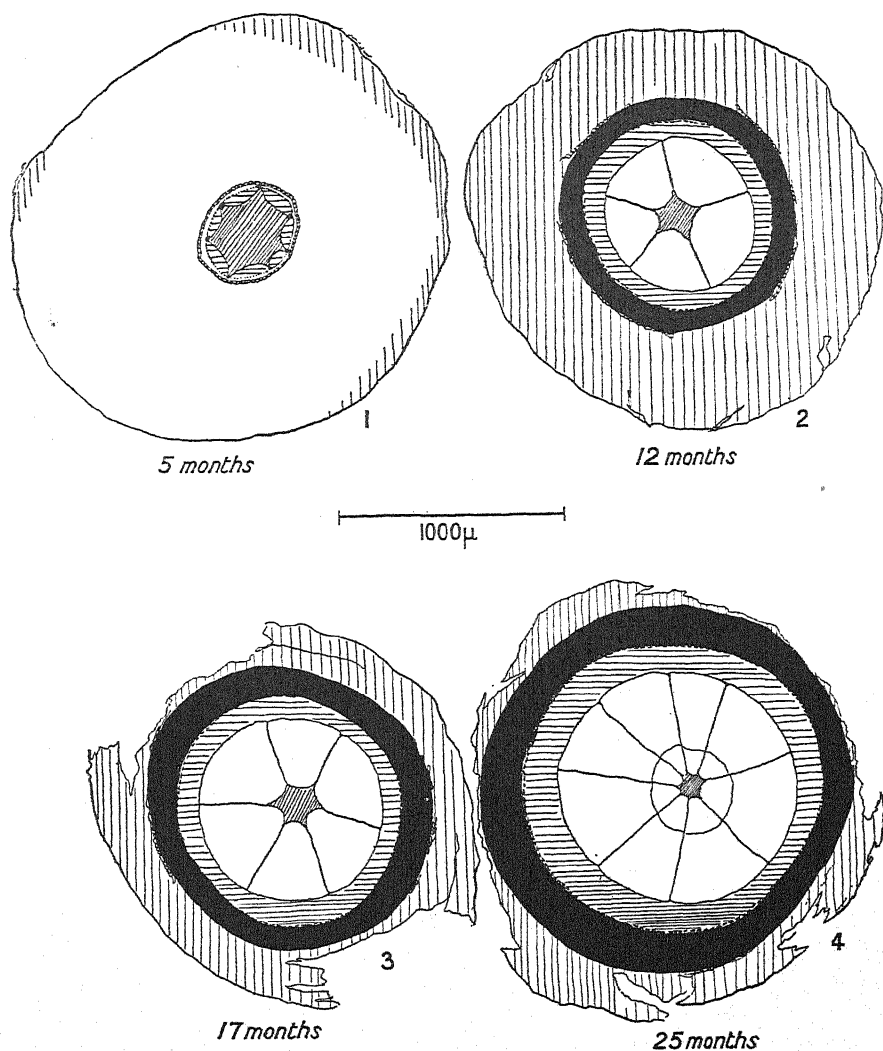
Tissue.	5 Months. December 1924.	12 Months. July 1925.	17 Months. December 1925.	25 Months. September 1926.
Cortex	94.4 ± 0.6	Dead	Dead	—
Polyderm and Pericycle	0.7 ± 0.04	36.0 ± 0.12	36.7 ± 0.20	36.0 ± 0.09
Phloem and Cambium .	1.4 ± 0.11	24.9 ± 0.27	20.7 ± 0.40	23.1 ± 0.27
Wood and Medulla . .	3.4 ± 0.15	39.0 ± 0.30	42.6 ± 0.37	40.9 ± 0.18
Total Stele	4.8 —	100.0 —	100.0 —	100.0 —

V. THE RELATION OF ROOT AND RHIZOME (CROWN).

In tracing the origin of the roots, it was found that their initiation occurred at a very early stage in the development of the shoot or crown before the differentiation of the interfascicular cambium. Consequently there is a very definite medullary connexion between root and shoot from the earliest stages of development. It has previously been recorded, both here and elsewhere (20), that the differentiation of vascular tissues in the very young root is slow. It frequently happens that the root has penetrated the endodermis, cortex and the soil to a distance of 3 or 4 cm. before vascular differentiation is observed. Xylem and phloem strands are first observed in the region of the root bounded by the ruptured cortex and leaf-base of the shoot. Ultimate connexion with the primary wood and phloem of the shoot is established rapidly by means of connective tracheids and parenchyma. The pericycle of root and shoot are continuous from the outset.

The development of secondary tissues is simultaneous in both root and shoot in the region of union. The vascular elements derived from the root cambium, since they lie in a plane at right angles to those developed from the vascular cambium of the shoot, form clearly-defined regions of the total vascular tissue seen in a transverse section of the lower portion of the rhizome. Phellogen development from the pericycle of rhizome and root is continuous, and subsequent decortication follows essentially the same course in both organs. The wide zone of muriform polyderm tissue, the unsuberized cells of which contain large reserves of starch at certain seasons, follows a very similar course of development in root and rhizome. The suberized cell cycles are, however, less numerous in the polyderm of

the rhizome, and free fatty substances are more abundant. An early stage in the development of the polyderm of the rhizome is shown in Pl. IX, Fig. 8.



TEXT-FIG. 14. Diagrammatic representation of the relation of the various tissues in roots of the Strawberry of different ages (see Table I in the text). Shaded: Vertical = Dead tissue; Horizontal = Phloem; Oblique = Medulla. Black: Polyderm. Unshaded: Xylem.

At the point of union of a root and the rhizome, the former usually shows a wide medulla of mixed parenchymatous, lignified cells. This tissue is continuous with the large medullary region of the rhizome. The parenchymatous cells of the whole region contain starch in abundance, and

seasonal fluctuations in the amount present occur. Accumulation and depletion take place slightly in advance of the same phenomena as observed in the more remote regions of the root.

VI. CHANGES IN THE ROOTS OF THE STRAWBERRY FROM DORMANCY TO THE FLOWERING PERIOD.

It has been shown elsewhere (9), that in the early growth phase of the plant, there is a very marked decrease in the total dry weight of the roots. It is further shown that this decrease is not due to actual loss in total bulk, in fact there is a slight increase in this direction, but is occasioned by a considerable decrease in the total dry matter constituents of the root system as a whole. A summary of the results obtained from dry matter determinations on the total root systems of groups of ten plants at different periods is given in Table II.

TABLE II.

Quantitative Changes in the Root Systems of Strawberry Plants set out on August 8, 1925.

Date.	Total Fresh Weight of Roots in Grm.	Total Dry Weight of Roots in Grm.	Percentage Dry Matter in Roots.
1925			
December 1	159.5	31.9	20.0
1926			
January 11	104.5	30.0	28.7
February 17	173.1	32.1	18.5
March 16	161.2	29.5	17.3
April 28	217.4	40.0	18.4
June 7	142.4	28.5	20.0
July 19	160.4	47.1	29.4
August 18	225.4	55.8	24.8
September 14	218.9	56.0	25.6

To study these changes still further, microchemical examinations were carried out on plants grown in pot cultures under different conditions. In July 1925 strong 'runners' were allowed to develop in small pots placed around healthy one year old parent plants. When the young root system was sufficiently developed, the stolon was severed, and the young plants were transferred to larger pots of 8 in. diameter. One hundred plants, each of two commercial varieties (Royal Sovereign and Duke), were prepared in this way. In the second week in November half of the plants, fifty of each variety, were transferred to a warm (65–70° F.) greenhouse, the rest remained in the open, suitably sheltered, and plunged in an ash bed.

By the first week in January the plants in the greenhouse had fully expanded flowers, and on January 18th many of the flowers had set fruit. The forced plants at this stage had the appearance exhibited towards the end of May by normal plants grown in the open.

Anatomical and microchemical examinations of the roots and rhizomes of the forced plants, and of the plants remaining in the open, were made at approximately weekly intervals from the beginning of December. The sequence of structural and chemical changes observed in normal, field-grown plants between November and May, was observed in the period from November to the end of January in the plants forced into rapid growth in the greenhouse. The plants from the pots in the open differed little from the normal field plants in these respects, and exhibited the features described for the normal plant during the dormant season. It is hardly necessary to describe in detail the various stages of developmental changes in the forced plants, a comparison of these with dormant plants in January will suffice.

A number of forced plants, and an equal number of the unforced dormant plants, were carefully washed free of soil. It was at once evident that the root systems of the forced plants showed the same features externally as the root system of a normal plant much later in the season. The older 'runner' roots were dark brown, and the main roots formed later in the Autumn were yellowish brown. The fine lateral roots borne on the main roots were very profuse and also yellowish brown in colour, contrasting markedly with the less branched, white to slightly yellowish brown, roots of the dormant plants. The colour difference is shown as a tone difference in the photograph in Pl. IX, Fig. 9.

Difference in internal structure and cell contents were equally marked. The older roots formed in July of the previous season showed secondary tissues in both cases. Death of cortex was further advanced in the flowering plants, and starch was absent from the cortical cells in both groups. The polyderm of the few roots of this type found on the dormant plants contained abundant starch, whilst in the case of the flowering plants, the majority of the 'runner' roots of which showed well-developed polyderm, disappearance of starch had taken place to a great extent (Pl. IX, Figs. 10 and 11).

The younger roots found on the dormant plants showed the primary structure, and the bulky cortex contained abundant starch. Roots still retaining the primary structure were of rare occurrence on the forced plants; the majority showed the beginnings of polyderm formation, and the bulky cortex was devoid of starch (Pl. IX, Figs. 12 and 13). The contrast between the younger roots of plants of the two groups was very striking in this respect. Tests with fat stains showed that suberization of the first formed cork cycle in the polyderm was well marked in the majority of the younger roots of the forced plants. The endodermis showed heavy suberization,

and the walls of the innermost cells of the cortex, adjacent to cells of the endodermis cycle, were often markedly suberized.

VII. DISCUSSION.

1. *The origin of adventitious roots.*

In horticultural practice the origin of adventitious roots is of especial interest and importance, in view of its intimate relation to vegetative propagation. From their hybrid or mutant nature, so many plants of economic importance cannot be grown true to type from seed, that some vegetative means of increasing their numbers must be employed. Some of these plants, such as the Strawberry and various species of the genus *Rubus*, produce specialized shoots which develop adventitious roots readily, and may be severed from the parent plant to continue a vigorous separate existence. Others, such as various members of the genus *Ribes*, can be propagated by means of cuttings, severed dormant or growing shoots, which readily produce adventitious roots under suitable conditions, and continue to grow vigorously. On the other hand, many species rarely develop adventitious roots, and are propagated with difficulty, or not at all, from severed portions of a parent plant. In these cases budding or grafting methods are employed, by which means advantage is taken of the meristematic properties of the vascular cambium to unite a portion of the shoot of the desirable species to a suitable foreign root system.

The anatomical and physiological factors determining the development of adventitious roots and shoots have been discussed by Priestley (13) in the light of recent researches, on the physiology of normal plant development. It is stated that 'an extended study of propagation in the dicotyledon leads to the generalization that the vascular cambium gives rise to root initials only, and the phellogen to new shoot initials', and 'when a Dicotyledonous shoot is used as a cutting, successful propagation depends upon the production of new roots from the cambium'. It has been shown, in the case of the Strawberry, that the adventitious roots are recognizable as small isolated groups of meristematic tissue lying between the pericycle and the medulla, at a stage of development preceding the formation of vascular cambium (Pl. VIII). At a slightly later stage the differentiation of an apical root meristem within the now enlarged meristematic group, may be distinguished in certain cases (Text-figs. 7 and 8), whilst in others it appears that further development of the initial group of meristematic cells is delayed. The meristems which develop most rapidly are those which occur near the vascular strands which form the leaf-trace bundles. This in part accounts for the observation made by previous workers (17), that the adventitious roots arise close to the leaf-trace gaps in the vascular cylinder of the stem. At this stage fascicular cambium is observed in process of differentiation

within the three vascular strands which form the median and two lateral traces to the leaf. These features have been constantly observed in tracing the origin of the adventitious roots in the youngest stages of 'runner' formation, and in the subsequent further production of roots as the rhizome (or crown) increases in age. It has further been noted that in the case of the 'runner', in the region of departure of vascular traces to the leaf or bract, numerous root initials occur opposite to the median leaf-trace, and between the two lateral traces (Text-fig. 3). Between the bases of these developing roots small xylem elements, not yet lignified, can be distinguished forming a much broken central vascular cylinder further interrupted at the leaf-trace gaps. Differentiation of vascular tissue in the stele of the 'runner' is accompanied by a similar differentiation of vascular tissues behind the growing points of the rapidly developing roots, so that a direct vascular connexion between root and shoot is early established, and the medulla and pericycle tissues of both are continuous from the outset. In the case of the isolated groups of meristematic tissue, in which further differentiation is delayed, the development of interfascicular cambium is observed immediately between these groups and the medulla. Occasionally the innermost cells of the meristem group become involved in the differentiation of this cambium. It often happens, especially just after transplantation of the 'runner' in which damage occurs to roots already well developed, that these quiescent meristems develop rapidly, and further adventitious roots are produced in the period shortly after replanting. In these cases the cambium cells immediately below the meristem elongate rapidly in the centre, and a direct connexion of the medulla of root and rhizome is again established, since the central radial region of the interfascicular cambium does not produce lignified elements, but only the parenchymatous tissue of the medullary ray it traverses. At the edges of this region the cambial cells of the rhizome become continuous with the secondary cambium of the vascular cylinder of the root. Examination of a young crown at this stage, when the first roots are half developed and new developing root primordia are observed in close proximity to the interfascicular cambium opposite to medullary rays, gives the impression that the adventitious roots have their origin from the interfascicular regions of the vascular cambium. It has been shown in the Strawberry that such is not the case, the origin of the adventitious roots can be traced to a much earlier stage before the initiation of vascular cambium. The probability exists that the groups of cells, which may give rise to adventitious roots, lie between pericycle and medulla, alternating with the primary vascular strands, and retain their meristematic nature from their origin at the broad apical meristem.

In an examination of the development of roots from the stems of *Clematis* spp., Smith (15) states that adventitious roots arise from the cambium opposite the medullary rays. Vochting (18) describes a similar

origin for the adventitious roots of the willow (*Salix fragilis*), and Priestley (13) describes the same phenomenon in the Blackberry (*Rubus leucostachys*). The writer has observed the same apparent origin of roots in shoots of the Black Currant (*Ribes nigrum*), Poplar, and several other *Salix* species. In all these cases the roots have been observed, either as latent growing points, or in various stages of later differentiation in close association with the cambium of stems in which considerable secondary vascular development has already taken place. In the case of the willow-root, young root initials have been observed at earlier stages of development lying on the same circumference as the fascicular cambium of the vascular bundles before the interfascicular cambium is distinguishable. Roots have also been developed from the leaf-bases of half-mature leaves of *Salix purpurea*, detached from the young shoots, and grown in narrow tubes with their petioles resting in culture solutions. In this case roots have their origin between the leaf-trace bundles in line with the union of phloem and xylem, which is not regularly marked by the presence of fascicular cambium in the case of the vascular traces of the leaves. It is suggested that in other cases further investigation of the stems of woody plants, such as those enumerated above, may reveal early stages in the development of root initials, where the association with a vascular cambium is less apparent.¹

The mechanical structure of the shoot may prove unfavourable for the further development of adventitious root initials, as shown by Smith (15) in the case of *Clematis*. It is further shown, in support of this suggestion, that by etiolation, which results in a general reduction of hard mechanical tissues, conditions are rendered favourable for further development of latent roots. Etiolation of shoots has been shown, in certain cases, to create within the stem, conditions comparable in some respects with those obtaining in the root (13). A well-marked endodermis may be produced, and it is suggested that its effect, in preventing diffusion of food materials outwards from the stele, is to create the necessary conditions for the development of root meristems within. With this hypothesis Smith is not in agreement, and suggests that the physiological changes, coupled with the modification of mechanical tissues, and brought about by etiolation, are the more important factors in creating the necessary internal conditions for the development of root meristems. It was observed by her that starch disappearance accompanied the reduced deposition of polysaccharide material in mechanical tissues in an etiolated shoot still attached to the parent plant. The growth of root meristems in the etiolated region is attributed to the fact that this diminution of excess carbohydrate material, whilst no interruption in the nitrogen supply occurs, produces the necessary chemical conditions for

¹ The writer is indebted to Mr. H. P. Hutchinson of this Station for this observation and for the use of preparations of young stems of various varieties of willow showing development of adventitious roots.

regeneration and development of meristematic tissue. In the case of *Clematis* etiolation does not result in the production of a clearly defined endodermis.

Later work on the rooting of stems and cuttings has recently been reviewed by Knight (6). There is a general consensus of opinion that the conditions obtaining within the shoot profoundly affect the production of adventitious roots. In the case of plants, such as the Tomato, which produce adventitious roots readily from stem cuttings, it is shown that a high carbohydrate content of the tissues, in relation to the nitrogen present or supplied, favours the development of adventitious roots.

In the present investigation no chemical analyses of the tissues have been carried out in relation to the study of root formation. The results of microchemical examination of the tissues, chiefly those in relation to starch, in which form the bulk of the carbohydrate reserves of the Strawberry are accumulated, in conjunction with the results of determinations of the changes in the percentage of the dry matter of the plant may be of interest. It has been shown that there are two well-defined periods of new root development in the annual growth cycle of the Strawberry plant. The first begins in the latter half of July, and is characterized by the development of vigorous adventitious roots, the second begins in early spring and consists in the development of numerous lateral roots from the main adventitious roots developed, in the first period. Both these periods of new root formation follow on periods of starch accumulation, and a corresponding high percentage of dry matter in the plant as a whole. The development of roots at both periods is accompanied by a rapid disappearance of accumulated carbohydrates, and a marked decrease in the percentage dry matter of both roots and rhizomes. It does not necessarily follow, however, that because the development of roots is observed externally in association with these particular conditions, that the same conditions obtain at the time of internal initiation of the root meristems. The possibility exists that the initiation of the group of meristematic cells, destined to develop into a root, may take place under conditions differing widely from those which obtain at the time when development outside the stem is observed.

The new adventitious roots which appear in July, arise in close proximity to the older leaf-bases. It has been found that groups of meristematic cells which may ultimately show differentiation into root initials, are constantly observed in the region of departure of the leaf-trace bundles in the early stages of growth (Text-fig. 10). It follows that the adventitious roots first observed in July, near the bases of old leaves, at a period in which microchemical tests show an accumulation of carbohydrates, were initiated at an earlier stage of the growing period, characterized by absence of stored carbohydrate material and the presence of a newly formed and efficient absorbing system of numerous lateral

roots. The immediate post-fruitle period is characterized by a development of foliage and young stolons, but little increase in root-growth occurs. In the young 'runner' the region of early initiation of the adventitious roots contains no starch, neither does accumulation of carbohydrate materials occur before the rapid development of the 'runner' roots takes place. Up to the time of establishment of these roots, however, the 'runner' is still an integral portion of the parent plant connected to the main body by a stolon, the anatomical structure of which shows it to be specially adapted for rapid translocation (20), so that the materials necessary for further development of the young root meristems are readily available.

The origin of lateral roots from the main adventitious roots is subject to the same general conditions which appear to determine the formation of adventitious roots in the tissues of the rhizome. In the period of rapid elongation the developing meristems of future lateral roots are observed in transverse sections of the main root. The accumulation of carbohydrate materials in the tissues of the main root takes place with the decrease in growth rate, and as this only occurs in the majority of the main roots with the approach of the dormant season, further development of the lateral roots is arrested until the first weeks of the following growing season.

Under certain conditions adventitious roots have been observed developing from the nodes of inflorescences of the Strawberry. Well-developed 'runner' plants, in the confined conditions of the 'runner' bed, cease to grow vigorously at an early date in the season. Transplanted to new beds in early August, active growth soon recommences, and vigorous new adventitious roots appear some three weeks after transplanting. Frequently abnormal inflorescences appear at this stage (Pl. X, Fig. 14) with short, thickened main and branch axes, and markedly swollen nodes within the sheathing bracts. Occasionally the primary flower expands in which the stamens are abortive, the pistils, though present (4), are much hypertrophied and the petals are pale green. From the swollen nodes normal adventitious roots make their appearance, and examination shows their origin to be essentially the same as that of normal 'runner' roots.

The cessation of growth in the crowded 'runner' bed is accompanied by an accumulation of carbohydrates in the roots and rhizome of the young 'runner' plant, and this accumulation continues to take place, for a short time after transplantation to a more open situation before new adventitious roots are developed. It has been shown that carbohydrate accumulation in excess of the nitrogen supplied by a vigorous absorbing root system, favours the development of flower rudiments (4, 8). With the growth of new roots and consequent increase in the nitrogen supply, the conditions necessary for vigorous vegetative growth are again established. The form

of reproductive organs, already partially differentiated under conditions of high carbohydrate content, cannot be changed, but their subsequent development may be modified in a vegetative direction. The root initials present at the nodes of the first inflorescence branches, in the region of departure of the vascular strands to the bracts, may continue further differentiation under these changed conditions.

Loree (8) has shown that increasing the nitrogen supply in the early phase of spring growth, results in greater stolon and new root formation in the summer. A further increase in the nitrogen supply to Strawberry plants in the immediate post-fruiting period, results in increased vigour of the second growth phase when the new adventitious roots are formed, so that the performance of the plants in the following fruiting period is markedly superior to that of untreated plants, or plants which received additional nitrogen in the spring only. The effect of nitrogen in stimulating vegetative growth in the spring is to maintain greater vigour in the plants during the exhaustive period of flowering and fruit setting. A similar result has been obtained (9, 10) by removing the flowers at an early stage. The greater vigour of the plants shown by the increased production of foliage and lateral roots, provides for more vigorous production of stolons and new roots later in the season. A further supply of nitrogen at this stage is available to the rapidly developing new adventitious roots, and vigorous growth is thus maintained. Increase in the number of leaves in a period of rapid growth is naturally accompanied by increase in the number of root initial groups within the stele of the rhizome; increased total area of the foliage provides for the carbohydrate supply, the accumulation of which is necessary for the further development of the root system.

2. Secondary development.

In a recent communication (20) White states that the early origin of the adventitious roots of the 'runner' of the Strawberry may be traced to the pericycle. Tangential divisions of certain cells in this tissue, after it has attained the multiple condition, shown to result from the formation of a phellogen, is stated to give rise to root initials, and ultimate connexion of vascular tissues of root and stem is established by tracheidal cells developed in the intervening pericycle tissue. In other words, adventitious roots have their origin in the tissues derived from a specialized phellogen which itself establishes vascular connexion. No evidence in support of this has been found in the present investigation.

It is further stated that in most cases the main adventitious roots of the Strawberry variety investigated, did not undergo secondary thickening, and therefore the vascular tissues of the root do not directly connect with the secondary vascular elements of the rhizome. In the present study secondary development in the main adventitious roots has been found to be

of general, rather than of exceptional occurrence. It is observed in the case of the first formed 'runner' roots, that secondary development of the root at the region near the union with the rhizome is frequently of early occurrence. In later-formed adventitious roots, secondary developments are delayed until the following spring. From this period onwards, through three consecutive growing seasons, harmonious development of secondary tissues, xylem, phloem, and polyderm is observed in the roots and the rhizome, and is continuous through the region of their union. Intimate connexion of the secondary tissues of root and shoot is a distinct and physiologically important structural feature of the mature Strawberry plant. It is suggested that the findings of White are based on the investigation of plants in a young stage of development before they had developed their full complement of secondary meristematic tissues.

The development of secondary elements from the vascular cambium of the Strawberry root appears to follow a normal course. The development of secondary phloem is small, and the formation of numerous secondary medullary rays are characteristic features of the root structure. The development of the specialized muriform polyderm from the pericycle phellogen presents features of considerable interest. A comprehensive study of phellogen and cork-formation has been made by Sanio (14) who is quoted by De Bary (3), and Solereder (16), and on whose original work much of the present knowledge rests. A phellogen may be differentiated in almost any tissue between the epidermis (*Pyrus Malus*), cortex (*Populus*), pericycle (*Ribes*), or even in the phloem in the bark formation of trees (*Quercus*, *Betula*). Almost any cell between these limits possessing living protoplasm and a nucleus can take part in the formation of the phellogen. According to Turpin (3), in the roots of herbaceous plants the sub-endodermal cells, i. e. the outer layers of the pericycle, are the usual seat of phellogen initiation, and this is observed in the present study. Cells cut off externally become suberized as a rule, forming a muriform cork (phellem) tissue, cells cut off internally form secondary pericycle tissue, but in roots the extent of this tissue is usually very small, being confined to one or two successive divisions of the phellogen cycle. In the external muriform tissue it frequently happens that zones of true cork alternate with zones of unsuberized cells. For these unsuberized cells Von Höhnell (5) has suggested the term phelloid cells. He states that frequently these phelloid cells differ in shape and form from the normal cork tissue, and often contain crystal inclusions of calcium or silica. They are of general occurrence in the bark of old trees where they form abscission layers between the plates (*Platanus*) or rings (*Betula*) of bark, along which cleavage occurs as outer layers of bark peel off with the increase in growth of the stem.

The formation of internal cork is of common occurrence in the natural order Rosaceae (12), particularly in the herbaceous genera *Potentilla*,

Agrimonia, *Geum*, *Fragaria* and *Poterium*. Weiss (19) states that in members of these genera 'the tissue produced by the cork cambium also includes cellulose cells (phelloid cells (5)), which are occasionally even more numerous than the suberized cells'. The condition obtaining in the case of cultivated varieties of *Fragaria*, described in the foregoing, is clearly an extreme of that described by Weiss. The bulk of the cells cut off by the phellogen are phelloid cells, and only a comparatively small number of cells become suberized. For this reason the writer has used the term polyderm in respect of this tissue, in preference to the terms cork and cork cambium. In a more recent study Mylius (11) describes the mode of origin and subsequent anatomical development of the polyderm in the genus *Fragaria*, and the features described by Mylius, and later by White (20), agree in all essentials with those observed in the present study. So far as the writer is aware, investigation of this highly specialized tissue from the physiological aspect has not previously been attempted.

As a purely protective tissue, such as that commonly produced by the phellogen, this secondary tissue in the Strawberry appears to be too highly organized and insufficiently suberized. The comparatively large bulk of the phelloid cells with, at certain seasons, abundant starch content, point to the specialization of this tissue for storage. Throughout the development of the root of the Strawberry, structural features appear to bear a close relation to this special physiological function. The bulky cortex of the primary root, with its abundant starch content, is protected by marked suberization of the outer living cortical cells. Suberin deposition in the cells of roots outside the endodermis is unusual, and it is even stated that fatty substances never reach the surface and deposit upon it as a continuous layer (7). In the Strawberry root such a continuous layer is produced, and is sufficiently impermeable to protect the more delicate cortical tissues through the dormant period and the early growth period, whilst the heavy starch reserves are being translocated, and the early differentiation of the first layers of the polyderm tissue takes place. With the ultimate decay of the cortex, during the flowering and fruiting period, the protection of the living tissues devolves on the polyderm. It appears significant that the first tangential cycle of cells cut off by the phellogen should rapidly become suberized. By the time secondary increase in diameter for the stele has advanced sufficiently to rupture the endodermis, two or three similar cycles of suberized cells are present in the polyderm, and starch in small quantity may be seen in the alternating wider zones of phelloid tissue. An important feature of the structure is the occurrence of unsuberized cells in the suberized cycles, which by tangential division provide for a certain amount of expansion of established cork layers, and also establish direct connexions between the intermediate zones of starch-containing phelloid tissue. The mature structure is best appreciated if the cork cycles are regarded as perforated

cylinders, in which the perforations of one cylinder alternate with the perforations of the smaller one within and the larger without, and the spaces intervening are filled with unsuberized phelloid cells, communicating with each other through the perforations in the cork cylinders. In this manner a maximum of protection is afforded by a minimum of suberin deposition, and a considerable amount of parenchymatous tissue, constituting approximately one third of the total volume of the root, is available for the special function of carbohydrate storage. In the present study the original 'runner' roots have been shown to persist through three complete seasons, yearly increasing in diameter, and showing an advanced condition of polyderm specialization. During the transition period from dormancy to the period of early spring growth, the dry matter content of these roots falls from 36 per cent. to 20 per cent. of the total weight. This quantitative change is shown to accompany rapid disappearance of carbohydrate materials from the phelloid tissue of the polyderm, the appearance of free fatty substances in the cells towards the outer layers and suberization of the phelloid cells now devoid of starch. Since older roots of this type neither produce many new lateral roots, nor indeed develop any new secondary tissues during the early growth period, it must be concluded that the stored carbohydrates are drawn upon by developing meristems in other parts of the plant.

The continuity of the polyderm in root and rhizome, and the general uniformity of structure exhibited by this tissue in both stem and root, are correlated with uniformity of function. Though in the rhizome, protected by persistent leaf-bases and developing above ground, the cycles of cork cells are less numerous, the phelloid cells show a fluctuating starch content comparable in all respects with that observed in the older roots, which further strengthens the view of their physiological function as specialized storage cells.

The comparatively short life of the lateral roots presents features of further interest. White (20) has shown that the pericycle of the lateral roots does not attain the multiple condition, and in the present study phellogen development in lateral roots was observed in a very few instances, and then only at the bases of strong lateral roots in the region of junction with the main adventitious root. It is significant that these fibrous roots, which constitute the bulk of the absorbing system of the plant in the spring and early summer, should become functionless as soon as new roots are developed in the second phase of growth which follows the conclusion of the fruiting period. The now functionless fibrous roots, unprotected by a polyderm tissue, rapidly decay, and a new system of absorbing lateral roots is developed on the vigorous adventitious roots arising from younger portions of the rhizome, whilst the original main roots continue to increase in thickness. The chief functions of the main adventitious roots seem therefore to be, first to penetrate the soil rapidly, and constitute a frame-

work upon which the absorbing system of fine fibrous roots is built up to support the plant during the following growing season, and secondly, to develop specialized tissues and function mainly as storage and anchoring organs in the subsequent life of the plant.

VIII. SUMMARY.

1. The development of the 'runner' of the Strawberry plant is described with special reference to the origin of adventitious roots.

2. Adventitious roots are shown to arise from small groups of meristematic tissue within the stele, alternating with the primary vascular strands, and between the outer pericycle tissue and the medullary tissues of the centre of the stele. These groups of meristematic tissue are observed in greatest number in the region of departure of the leaf-trace bundles. From the observations made, it is suggested that cells destined to be the mother-cells of adventitious roots retain their meristematic nature from their origin at the growing point.

3. Differentiation of the apical meristem of the root occurs before the differentiation of the secondary vascular cambium of the stem in the majority of cases. In this respect the development of adventitious roots in the Strawberry appears to differ from that recorded in other plants.

4. Microchemical examinations of the tissues, together with determinations of changes in the dry matter content of the roots and rhizomes have been made at regular intervals through two complete growing seasons. The results are discussed in relation to the observed phenomena of root development.

5. The initiation of root meristems in the stem occurs during periods of vigorous growth, characterized by the absence of excess carbohydrates and a low dry matter content of the tissues. Further development of the roots follows on a period of carbohydrate accumulation as indicated by deposition of starch in the storage parenchyma of the plant as a whole, and a marked increase in dry matter content.

6. When vigorous growth of the shoot occurs simultaneously with a readily available supply of carbohydrate materials, as in the case of the young 'runner' plant, further development of root meristems follows closely on their initiation.

7. The anatomical structure of the root has been followed through the greater part of three seasons, from the date of origin from the 'runner'. The development of a specialized polyderm forms a very characteristic feature of the older main roots, which continue to develop secondary tissues and function little as absorbing organs after their first season's growth.

8. It is shown that the structure of the lateral roots differs essentially from that of the main adventitious roots. The bulk of the lateral roots are

developed in spring, no polyderm development is observed in the majority of cases, and they become functionless to a great extent with the formation of new adventitious roots in the later summer and autumn.

9. Microchemical study of the tissues of the main roots at regular intervals during the season, shows that considerable fluctuations in carbohydrate content occur. In the primary condition of the root, starch accumulates in the cortex; in roots showing secondary developments of phellogen and vascular tissues, the phelloid cells of the polyderm form the chief region of starch deposition. Accumulation occurs as the dormant season is approached, and again during the month of June and in early July. Depletion takes place in the spring and in late July, during periods of vigorous new root development.

10. The specialized structure of the polyderm is described in detail, and its organization as a combined protective and storage tissue is discussed. Its continuity in root and rhizome is demonstrated.

ACKNOWLEDGEMENTS.

The writer wishes to express his indebtedness to Mr. E. Ball, in collaboration with whom the previous studies have been carried on from which this investigation arose; to Professor Barker for his unfailing interest and many helpful suggestions, and to Mr. F. E. Maltby for assistance in the preparation of numerous serial sections.

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DESCRIPTION OF PLATES VIII-X.

Illustrating Mr. C. E. T. Mann's paper on the Origin, Development, and Function
of the Roots of the Cultivated Strawberry.

PLATE VIII.

Fig. 1. Transverse section of the stolon at the point of origin of the runner (approximately 5 mm. behind the growing point) showing early stage in the development of an adventitious root. *c.*, cortex; *e.*, endodermis; *m.*, young root meristem; *px.*, primary xylem. Note absence of secondary cambium at this stage. Scale = 300 μ .

Fig. 2. Transverse section of root showing early stage of phellogen differentiation. Section stained with Sudan III. Note heavy suberization of the endodermis. *px.*, primary xylem; *p.*, pericycle; *pg.*, phellogen; *e.*, endodermis. Scale = 50 μ .

Fig. 3. Transverse section of a root fourteen months old. Stained with Sudan III. Note structure of the polyderm with its concentric cycles of cork alternating with the wider zones of phelloid tissue unstained. *p.*, polyderm; *c.*, dead cortex and endodermis. Scale = 500 μ .

Fig. 4. Detail of polyderm in Fig. 1—cork cycles. Note occurrence of unsuberized cells in the cork cycles. Fragments of the ruptured endodermis can be distinguished. *c.*, cork cells; *p.*, phelloid cells; *e.*, endodermis. Scale = 300 μ .

Fig. 5. Transverse section of root eighteen months old in the dormant season. Stained iodine. Note heavy starch content of polyderm layer. Outer cycle of polyderm tissue contains little starch. Scale = 500 μ .

Fig. 6. Detailed structure of the polyderm from a root comparable in age with that shown in Fig. 3. Stained 1 per cent. osmic acid. Heavy deposits of fatty substances present in outer layer of polyderm. Scale = 100 μ .

PLATE IX.

Fig. 7. Structure of the polyderm. Transverse section of polyderm tissue of root shown in Fig. 3. Stained with Sudan III. The granular appearance of the phelloid cells is due to their heavy starch content. Note presence of fatty compounds in the pericycle. *p.*, pericycle; *pg.*, phellogen; *pd.*, phelloid cells; *c.*, cork. Scale = 50 μ .

Fig. 8. Transverse section of rhizome showing structure of the polyderm. Stained with Sudan III. *e.*, endodermis; *c.*, cork cycle.

Fig. 9. Comparison of dormant plant with one of the same age grown for seven weeks from November to January under forcing conditions. Note darker colour of the roots of the forced plant and the larger proportion of fine lateral roots. Scale = 15 cm.

Figs. 10 and 11. Comparison of starch content of an older 'runner' root of the dormant plant (10) with that of the forced plant (11). Sections stained with iodine.

Figs. 12 and 13. Comparison of starch content of young roots of the two plants. (In Figs. 3, 4, 5, and 6 scale = 1 mm.) (In Figs. 10, 11, 12 and 13 scale = 1 mm.)

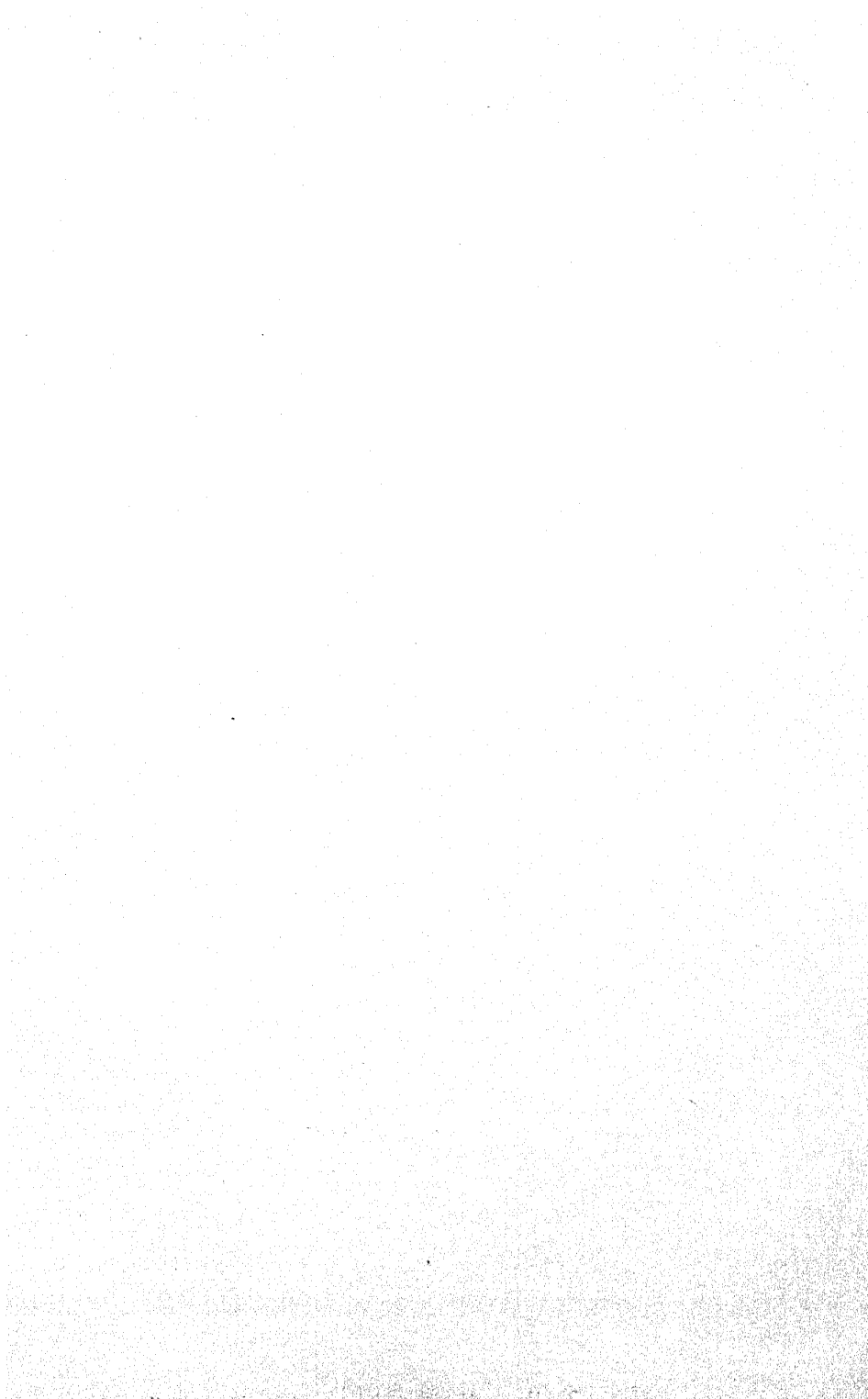
PLATE X.

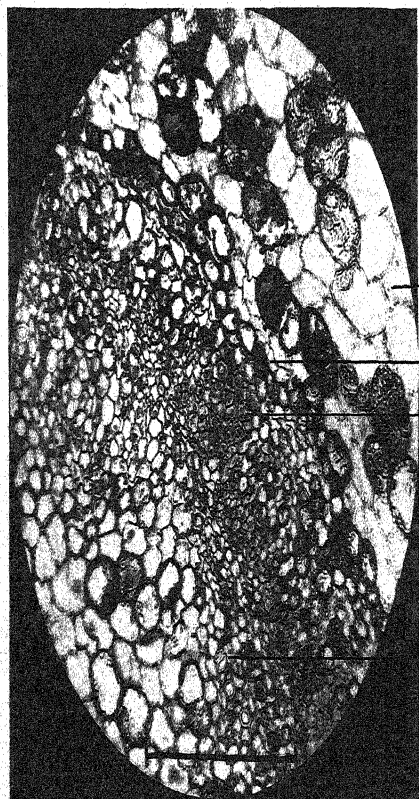
Fig. 14. Showing the development of adventitious roots from the nodes of an abnormal inflorescence produced under special conditions described in the text. Scale = 5 cm.

Fig. 15. Early development of adventitious roots from the young 'runner' of the Strawberry plant. Condition in July. Scale = 5 cm.

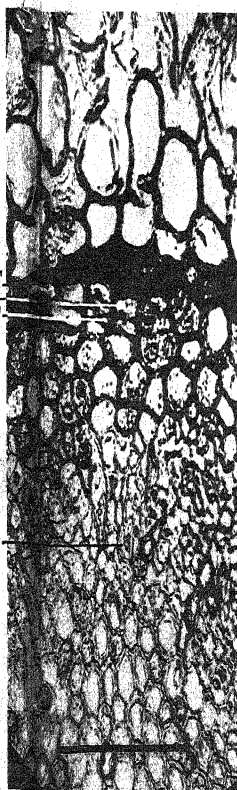
Fig. 16. The commencement of the development of adventitious roots in the post-fruited period. One year old plant in July. Scale = 5 cm.

Fig. 17. Showing the condition of a similar plant in December. Note the profuse development of vigorous, white adventitious roots. The older roots are dark brown or black. Scale = 10 cm.

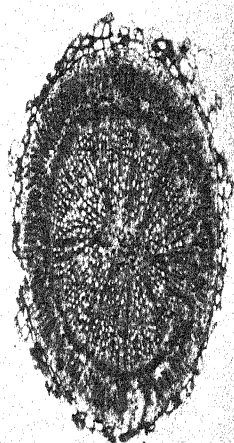




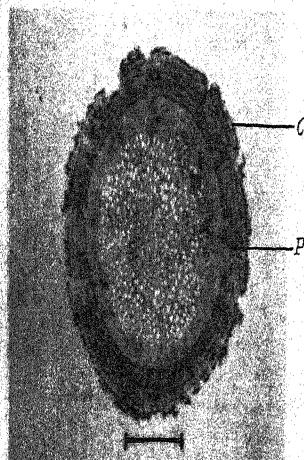
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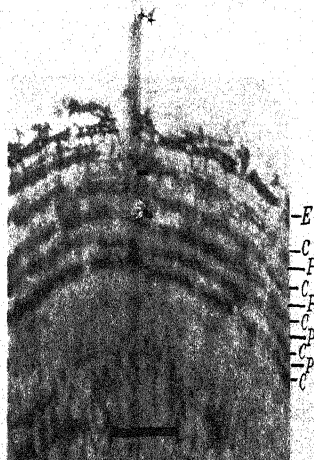
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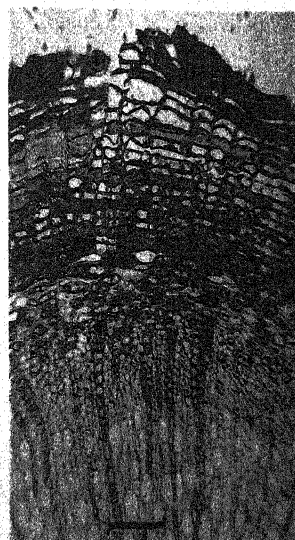
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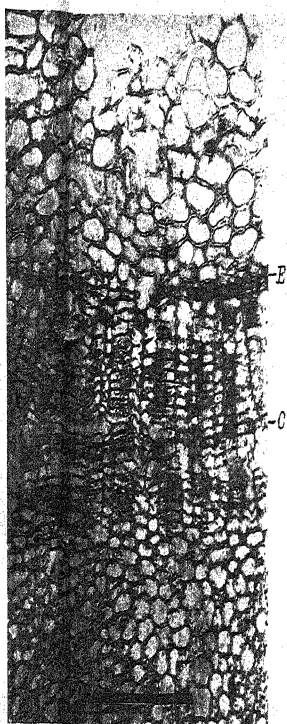
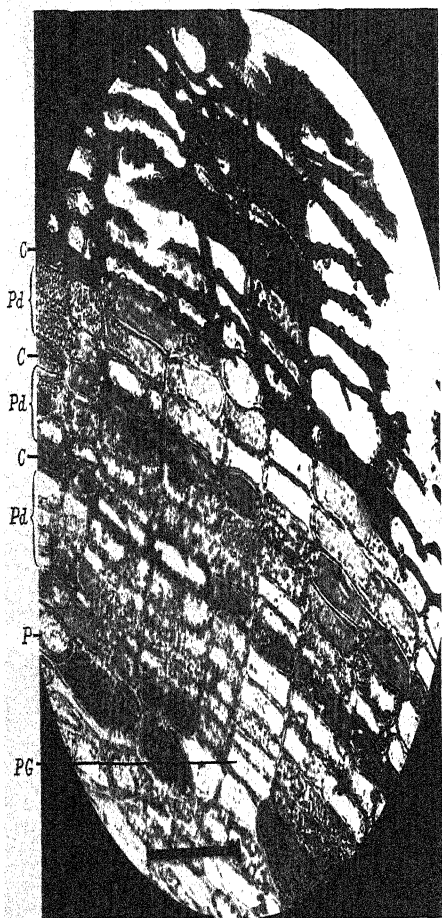
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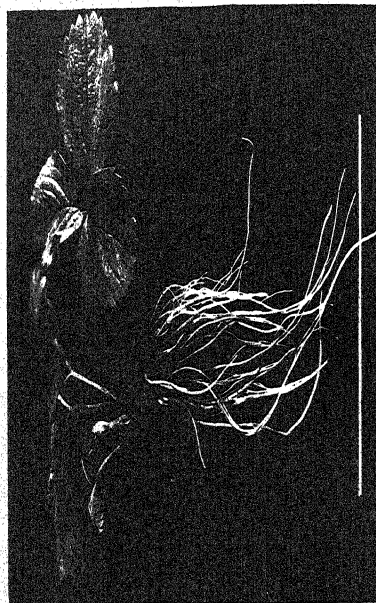
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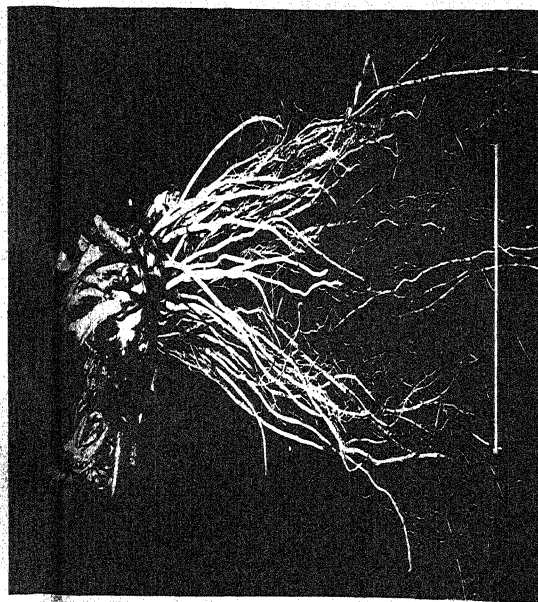
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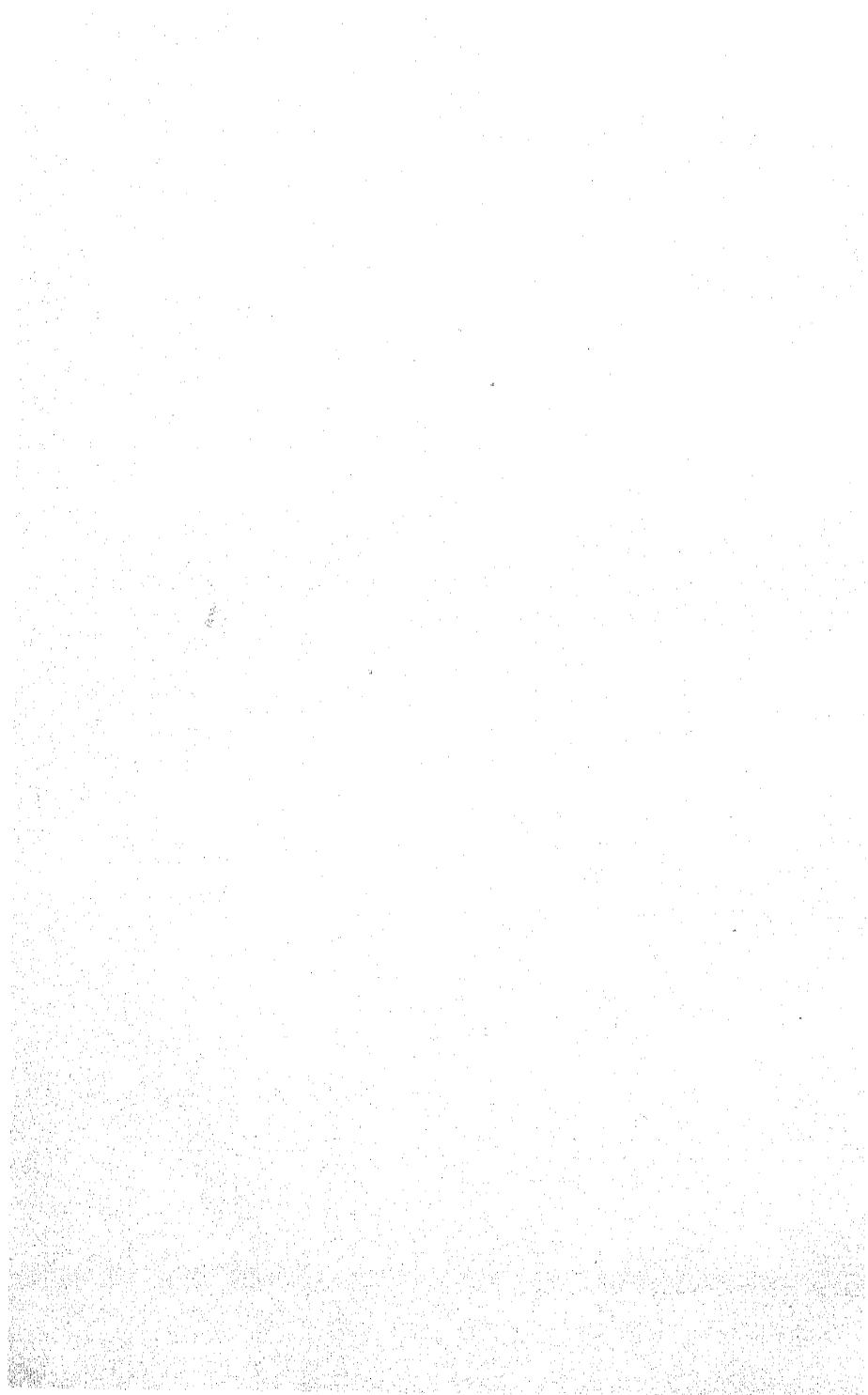
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MAEN — SHOOT DEVELOPMENT OF STRAWBERRY.

Hort. London



Cleistogamy in *Viola Riviniana* with Especial Reference to its Cytological Aspects.

BY

GERTRUDE WEST, M.Sc.

(*Royal Holloway College*).

With Plates XI and XII and four Figures in the Text.

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INTRODUCTION.

IT has been shown (16) that seed production in the cleistogamous flowers of *Viola odorata* depends upon a true fertilization. The present study of *V. Riviniana* was undertaken to determine the conditions in another *Viola* species and so help towards a generalization for the genus. Now the chasmogamous flowers of *V. odorata* are invariably sterile and only the cleistogamous ones set seed; whereas in *V. Riviniana* some seed is set by the open flowers as well. Thus, there is the corresponding problem of seed production in those chasmogamous flowers which set seed, but owing to the infrequency of seed production by these flowers, and the consequent scarcity of material, it has not been possible to investigate it. Fertilization is, therefore, described for the cleistogamous flowers only.

MATERIAL.

All the material used in the investigation was obtained from plants growing either in the grounds or in the botanical garden at Royal Holloway College. Three groups of plants were kept under observation, viz.:

1. Plants of *V. Riviniana* Reichb., blue and white forms, sent by courtesy of Mr. Hales from the Chelsea Physic Garden, which were grown in sun and shade plots in the botanical garden.

2. Two patches of wild plants, which may be described as follows:

- (a) *V. Riviniana* Reichb., growing among ivy and grass, well shaded by cedar trees above, receiving sun from south-west and west.

- (b) *V. Riviniana* var. *nemorosa* (N.W. and H.), also growing among ivy and wild arum, shaded by cedar and hawthorn trees, receiving sun from south-east and south.

These two groups of wild plants were not interfered with in any way, so that it was possible to study fresh material of the various types of flower as they actually occur in nature. Observations were begun on the plants from the Chelsea Physic Garden in the spring of 1926, and on the wild plants in the spring of 1927, and were continued until July 1928. These groups of plants proved very good sources of material for morphological and for cytological work. Occasional attacks of 'smut' caused by *Urocystis violae* seemed to have no effect whatever upon the life cycle.

METHODS.

The general form of the types of flower produced by the three groups of plants of *V. Riviniana* during the flowering season was studied as far as possible in fresh material. A calendar was kept recording the periods during which the chasmogamous, semi-cleistogamous, and cleistogamous flowers appeared. For the cytological investigation the buds and flowers were always fixed as soon as possible so that never more than ten or fifteen minutes elapsed between gathering and fixing; sometimes fixing was done in the field.

The following fixing fluids gave the best results:

1. Allen's modification of Bouin's fluid.
2. Flemming's fluid, weak solution.
3. Medium chrom-acetic.
4. Acetic alcohol (2:3).

Hermann's and Merkel's fluids were also used to a very small extent. Chloroform proved to be a very satisfactory and rapid clearing agent, the material being less brittle than when cleared in xylol. Sections were cut 8μ in thickness.

The following stains were used most successfully:

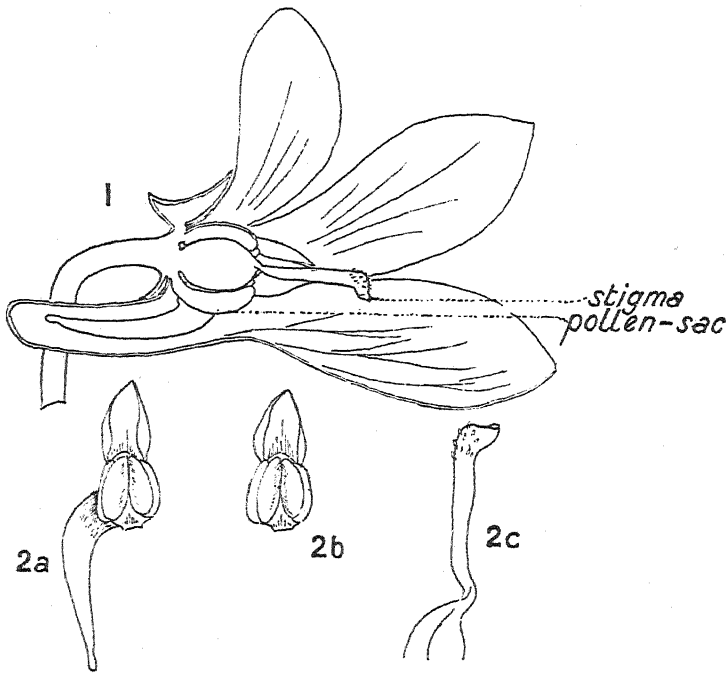
1. Heidenhain's iron-alum-haematoxylin, usually counter-stained with orange G, following Allen's Bouin fixative.
2. Gram's iodine, gentian-violet method.
 - (a) Clausen's modification (3) after Allen's Bouin fixative.
 - (b) Newton's modification (7) after Flemming's fluid and medium chrom-acetic.

Pollen smears. Smear preparations of the pollen mother-cells and very young pollen-grains of chasmogamous flowers of *V. Riviniana* were tried. Although the stamens are not favourable material for this method, being so small, some good preparations were obtained. Excellent fixation and staining were given by Flemming's fluid, weak solution, followed by Newton's iodine gentian-violet stain.

MORPHOLOGY.

Chasmogamous flowers. The general morphological features of the open flowers of *V. Riviniana* are represented in Text-fig. 1. The flowers show the grooved spur which distinguishes the species from *V. sylvestris*, with which it is sometimes confused. The spur of the blue flowers of *V. Riviniana* Reichb. is blunt and of dull whitish blue, whereas that of *V. Riviniana* var. *nemorosa* is quite a deep purplish blue, and this difference in colour is noted in the expanded petals.

Cleistogamous flowers. The morphology of the permanently closed flowers of *V. Riviniana* is particularly interesting. Cleistogamous flowers appear when the chasmogamous season has finished, and may therefore be called the summer flowers. The reduction of the floral members of these



TEXT-FIG. 1. Chasmogamous flower. 1. Flower partly dissected. 2a. Anterior stamen. 2b. Lateral or posterior stamen. 2c. Style.

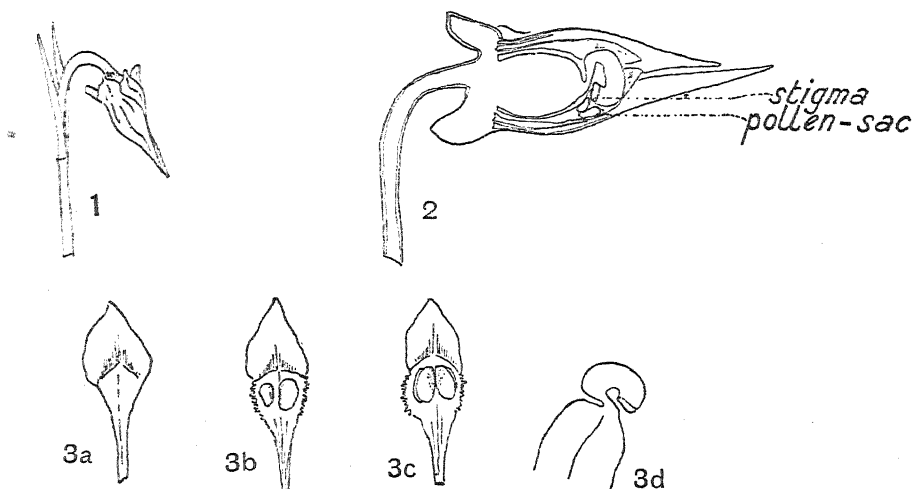
flowers is carried to what is almost the extreme limit possible: only those parts essential for the protection of the reproductive organs and for seed production are fully developed. The other parts are either absent or represented merely by rudimentary structures. A cleistogamous flower of *V. Riviniana* shows the following features.

Calyx. The five normal sepals enclose the flower very tightly and show no sign of opening until fruit formation.

Corolla. The petals are represented merely by two to five small membranous strap-shaped structures almost entirely unpigmented. In extreme cases the corolla is absent.

Androeceum. Considerable variation is found in the extent of fertility of the five stamens. They are all present, though very much reduced. The greater part of the tissue below the connective is sterile and appears as a long stalk-like structure. The anterior stamens have two to four pollen-sacs each, which, although less than one-third of the size of the pollen-sacs in

a chasmogamous flower, are the largest in the cleistogamous flower and are certainly the ones functional in self-pollination. The nectaries typical of the anterior stamens in open flowers are absent. The lateral stamens have either two or three pollen-sacs each, or there is only one ; occasionally one



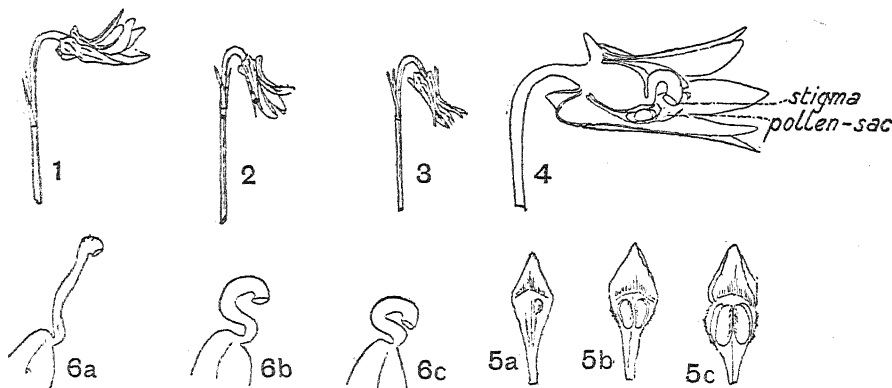
TEXT-FIG. 2. Cleistogamous flower. 1. Form of flower. 2. Flower partly dissected. 3a. Posterior stamen. 3b. Lateral stamen. 3c. Anterior stamen. 3d. Style.

lateral stamen is sterile. The posterior stamen is invariably sterile. The orange coloured connectives of the stamens are fully developed and arch like a roof over the curved style and stigma, providing excellent protection for the reproductive organs (Text-fig. 2). The cells of the epidermal layer of the pollen-sac wall show the characteristic thickenings on the inner and radial walls even in cleistogamous flowers in which dehiscence does not take place. The stamens are connected to one another by peg-like outgrowths developed on the contiguous edges of the outer pollen-sacs. These interlock in such a way that often, when a flower is dissected, all the five stamens come away together.

Gynaeceum. The ovary of a cleistogamous flower is normal, but the style and stigma are much modified. The style is bent over in the form of a hook, and by this curvature the stigmatic surface is brought over and down towards the pollen-sacs of the two anterior stamens. The stigma, as found in open flowers, is absent and the stigmatic surface is represented merely by the flattened end of the tubular style and is in close contact with the upper surface of the anterior pollen-sacs ; the stigmatic aperture is much larger than that in an open flower.

Semi-cleistogamous flowers. Between the full periods of chasmogamous and of cleistogamous flowers, a few flowers, designated semi-cleistogamous,

are produced, which, besides linking the open and closed flowers in regard to time of flowering, are found to illustrate, in a series of forms, the assumed stages in reduction from chasmogamy to cleistogamy. The fact that this process of reduction can be found occurring during one flowering season is evidence for the origin of the cleistogamous flower from the chasmogamous



TEXT-FIG. 3. Semi-cleistogamous flower. 1-3. Three forms of semi-cleistogamous flower. 4. Flower partly dissected. 5a. Posterior stamen. 5b. Lateral stamen. 5c. Anterior stamen. 6a-c. Three forms of style found in semi-cleistogamous flowers.

in such a way. The only distinction between the earliest semi-cleistogamous flowers and the chasmogamous flowers is merely that the petals of the former are considerably smaller than those of the latter. As the season progresses the semi-cleistogamous flowers show more and more indications of cleistogamy in the following features: reduction of the petals and of the number and size of the fertile pollen-sacs, and the curvature of the style and stigma. The latest of these flowers differ from a cleistogamous flower merely in that they show one or two small, slightly pigmented petals extending beyond the sepals. Text-fig. 3 illustrates three semi-cleistogamous flowers which show the gradual reduction of the petals and the closing of the flower. The change in the relative positions of the stigma and pollen-sacs, brought about by the curving of the style, may be followed by reference to Text-figs. 1 (Fig. 1), 2 (Fig. 2), 3 (Fig. 4). The following annual cycle of flower production may be deduced for *V. Riviniana* from the records kept from 1926 to 1928:

Chasmogamous flowers. March to middle of May.

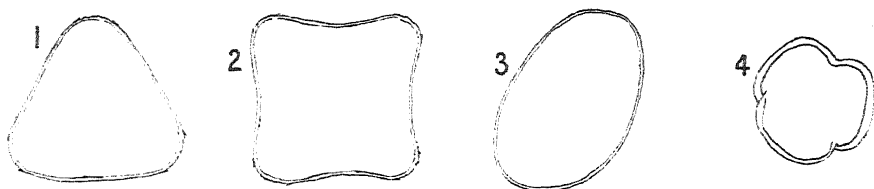
Semi-cleistogamous flowers. May.

Cleistogamous flowers. Middle of May to September.

POLLINATION AND SEED PRODUCTION.

Two kinds of pollen-grain are produced in the pollen-sacs of the chasmogamous flowers, and in addition there are many shrivelled pollen-grain walls. Of the good grains the majority are large, either tetrahedral

or oval, thin walled, and contain finely granular protoplasm. The smaller grains have thicker lobed walls, and the protoplasm is dense and lumpy. A small proportion of these small grains germinate within the anthers: the large grains are shed in the binucleate condition. The pollen-grains of cleistogamous flowers, many of which degenerate, are like the smaller



TEXT-FIG. 4. Pollen-grains of the chasmogamous flower. 1-3. Forms of pollen-grains which are shed. 4. Form of pollen-grain which germinates within the anther.

grains of the open flowers; the good grains germinate within the anthers. It is an interesting fact that these pollen-grains which germinate within the anthers of chasmogamous flowers are similar in form to those found in cleistogamous, and that the pollen-grains functional in cross pollination are morphologically, and probably physiologically, different from those which germinate within the anthers.

Cross pollination in the open flowers of *V. Riviniana* has not been made a part of the present study: the general features of the pollination mechanism in *Viola* are well known. The neat and direct method of self-pollination in the cleistogamous flowers has been described by other workers, and only the essential features are here recalled. The pollen-grains germinate within the pollen-sacs; the pollen-tubes so produced penetrate the anther wall at the apex of the sac, pass directly into the stigmatic orifice, thence through the stylar canal into the ovary. Pollen-tubes from the anterior stamens only are functional in pollination. The observations made on pollination in semi-cleistogamous flowers are scanty, but enough to indicate that the pollen germinates within the anthers, and the pollen-tubes traverse a considerable distance towards the stigma. The greater number of the pollen-tubes, however, fail to reach the stigma, and this probably accounts for the noticeable failure of seed production by semi-cleistogamous flowers.

Good seed is set by cleistogamous flowers, except towards August and September, when a considerable number of flowers die off without producing seed. The open flowers of the cultivated *V. Riviniana* set seed, but many open flowers of the wild plants fail to do so: the causes of this failure are not known. Throughout this work it was noticed that no capsules were ever produced by the flowers of *V. Riviniana* var. *nemorosa*. Dissection of mature and even faded flowers showed that the pollen was retained with the anthers in pollinium-like masses, one 'pollinium' in each

pollen-sac. The good grains, several of which were seen to have germinated, were bound together by shrivelled pollen-grains, and although the anthers dehisced normally the pollen-masses were not dispersed. A few pollinating experiments were carried out with these pollen-masses and one positive result was obtained. A pollen-mass from an anterior stamen of one of these flowers was transferred to the stigma of another. The flower was tied up in a pollinating bag, and after twenty days a good capsule had developed. This is considered to show that the pollen was not sterile, and that seed production failed because the pollen was not dispersed. Apparently pollen was never brought to these flowers from other *Viola* flowers.

CYTOLOGY OF THE POLLEN.

The stages described in the following account of the meiotic division in the pollen mother-cells of *V. Riviniana* were studied in microtome sections of chasmogamous buds. Some stages were observed in cleistogamous material, and as far as can be concluded from these the pollen development is similar in both kinds of flower. Those stages in pollen meiosis shown by smear preparations confirm the conclusions drawn from microtome sections and include resting nuclei, synizesis showing the variation in nucleolar form and the connexion of the thread to the nucleolus, the linking of the bivalent chromosomes prior to the heterotype division, as well as some stages between heterotype telophase, homotype telophase, and pollen-tetrad formation.

It was often found that the pollen mother-cells at the apex of a pollen-sac lagged behind the pollen mother-cells at the base as regards the stage reached in development. For instance, in one particular pollen-sac the stages from diakinesis to heterotype metaphase, or from interkinesis to homotype telophase could be followed from the apex to the base. This gradual progression of the stages throughout one pollen-sac enabled one to visualize more clearly the phases passed through in living cells. More prolonged phases, such as diakinesis, were often found throughout a whole pollen-sac. Again, the stages shown by the various pollen-sacs in one bud differed widely. In one case the anterior stamens showed first contraction to open spireme, the laterals diakinesis and heterotype division stages, and the posterior stamen homotype division and tetrad formation. Thus the anterior stamens are considerably later in development than the others. The chasmogamous flowers are decidedly protandrous, but in cleistogamous flowers the pollen and ovules must of necessity be mature at the same time. Since only the anterior stamens are functional in the pollination of cleistogamous flowers, the retardation of development in the corresponding stamens of chasmogamous flowers seems to be a definite adaptive tendency towards cleistogamy.

The following account of meiosis in the pollen mother-cells is practically confined to a description of those features which are apparently peculiarly characteristic of *V. Riviniana*. It may, therefore, be concluded that those details not described are quite typical of a meiotic division in the prophases of which two contractions are passed through.

Meiosis. Heterotype division. The large nucleoli of the resting pollen mother-cells are usually vacuolate and occasionally contain a small crystal body (Pl. XI, Fig. 1). Many of the nucleoli in the newly differentiated tapetal cells contain a similar but larger crystal body; in some nucleoli two or three smaller crystal bodies are present. During synizesis or first contraction, the nucleolus is flattened against the nuclear membrane, either close to the contraction knot or on the opposite side of the nucleus (Pl. XI, Fig. 2). Nucleoli of much altered and very peculiar shapes occur in many of the pollen mother-cells at this stage. The change in form seems to be brought about by a spreading of the nucleolar material against the nuclear membrane, accompanied by vacuolation, so that the nucleolus becomes flattened and much attenuated; it is almost plate-like in form. The outline of many such nucleoli is irregularly lobed. A similar change and variation in shape of the pollen mother-cell nucleoli during synizesis is recorded for *Lathyrus odoratus* (14). Throughout first contraction connexion is maintained between the nucleolus and the synizetic knot by a delicate thread or threads passing from the knot to the nucleolus. The point of connexion is usually marked by a small bulge on the surface of the nucleolus. In good preparations, especially when Newton's staining method is used, this bulge is deeply stained while the rest of the nucleolus is quite unstained (Pl. XI, Fig. 3). Since it is probable that this bulge is similar in position, form, and function to the 'nucleolar body' described by Latter for *Lathyrus* the same term is adopted here, although its origin and function are not so clearly defined as they appear to be in *Lathyrus*.

At diakinesis twenty bivalent chromosomes of various shapes are scattered within the nucleus. The univalent halves of the early bivalent chromosomes are quite separate, and delicate unstainable extensions are seen, probably of linin material, at the free ends of the univalents (Pl. XI, Fig. 4). In some nuclei similar threads appear to connect some of the bivalent chromosomes to one another (Pl. XI, Figs. 5, 6). The univalent chromosomes close together later forming compact bivalents with little variety in shape. At this stage a peculiar formation is noticeable in the nuclei: the bivalents are connected or linked together in groups, and it seems that they are connected by the linin threads mentioned above (Pl. XI, Fig. 7). In some nuclei this linking is very marked, and indeed the bivalents seem to be fused to one another, so that the limits of some chromosomes are indistinguishable. Much variation is found in the degree of this linking and in the number of groups of linked chromosomes formed in different nuclei. It is, therefore,

difficult to offer an interpretation of this unusual phase, since, although it seems to be a constant feature of pollen development immediately preceding the heterotype division, there is apparently no definite rule according to which the chromosome groups are formed, nor any constancy in the number of chromosomes in the groups in different pollen mother-cell nuclei. The gradual disappearance of the nucleolus during diakinesis may be correlated with the appearance of chromatin areas in the cytoplasm during heterotype telophase. In those nuclei which show clearly the stages of spindle formation a thickening of the nuclear membrane is evident during late diakinesis, possibly owing to the development of spindle fibres within the nuclear membrane (Pl. XI, Fig. 8). This disappears later and a tripolar spindle is formed, which afterwards becomes bipolar.

In some pollen mother-cells at metaphase, the chromosomes, still in their linked groups, form a ring at the equator of a 'hollow' spindle: in others the chromosomes form a plate at the equator of a 'solid' spindle. It is thought that the 'solid' spindle is not an alternative structure to the 'hollow' spindle, but rather that it is merely a later development. The two are, however, distinctly recognizable.

The period of interkinetal rest is quite long. The heterotype spindle disappears; two or three nucleoli are formed within each resting nucleus and about twenty minute beads of chromatin appear upon the linin reticulum; these may represent the heterotype chromosomes. The chromatin beads form the basis of chromosome formation during the homotype prophase.

Pollen-grain wall formation. At the beginning of the delimitation of the four microspores a narrow groove appears along each of four lines equidistant on the surface of the pollen mother-cell cytoplasm, and midway between the nuclei. This cytoplasmic furrowing is accompanied by an ingrowth of material from the thick pollen mother-cell wall along those lines, and a wedge of wall material fills each groove (Pl. XI, Figs. 9, 10). The furrowing and accompanying ingrowth of the wall continue until the four walls meet in the centre of the cell, and thus the four members of the tetrad are formed. A similar process of pollen tetrad-wall formation has been described for *Lathraea* spp. (9) and in *Lathyrus odoratus* (14). In pollen mother-cells in which furrowing has begun, delicate cell-plates are seen at the equators of the spindles (Pl. XI, Fig. 9). These possibly assist in the formation of the tetrad wall, although they appear to be merely evanescent structures. A thin wall is secreted around each microspore, and the thick tetrad wall disappears; it becomes spongy in appearance, and seems merely to dissolve in the contents of the pollen-sac (Pl. XI, Figs. 11, 12). The walls of the young free pollen-grains become thickened, and clearly show four tetrahedral lobes.

Development of the binucleate pollen-grain. A large central vacuole is

formed within the young uninucleate pollen-grain and the nucleus is pushed to the periphery. At the beginning of the nuclear division within the pollen-grain twenty long rod-shaped, or slightly curved chromosomes are formed within the resting nucleus. The daughter chromosomes which separate on the short spindle are V-shaped, and quite separate from one another. A cell-plate is formed during telophase, and although this soon disappears it may participate in the differentiation of the protoplasm around the generative nucleus. The lenticular generative cell is at first close to the wall of the pollen-grain; its nucleus is smaller than the vegetative nucleus, and when first formed each nucleus is composed* almost entirely of a single nucleolus. In the mature pollen-grains, especially of open flowers, the generative cell is no longer flattened against the pollen-grain wall, but has moved into the cytoplasm of the pollen-grain. It is long, narrow and spindle-shaped, and contains an oval nucleus with a stainable reticulum and small nucleolus. A small stainable body is sometimes observed in the protoplasm at one end of the generative nucleus (Pl. XII, Fig. 16).

Spermatogenesis and germination of the pollen. Spermatogenesis proceeds similarly in both chasmogamous and cleistogamous pollen-grains. The male-cells formed in the two kinds of flower of *V. Riviniana* are exactly alike, and very similar to those described by Madge (16) for *Viola odorata*: it may, therefore, be that this form of the male gametes is common to the genus. During the division of the generative nucleus of *V. Riviniana*, particularly during the later phases, several small stainable granular bodies appear on the spindle. At telophase they occur at the equator, and are distributed in approximately equal numbers between the two male cells (Pl. XII, Fig. 18). These granules undoubtedly give rise to or persist as the stainable 'dots' in the cytoplasm of each male gamete. Although it is not yet proved, it seems very probable that these granules originate, possibly by fragmentation, from the one larger stainable body observed at one end of the resting generative nucleus. Two pear-shaped male cells are formed at spermatogenesis, each containing a spherical or oval nucleus in the round end, and the stainable 'dots' in the cytoplasm of the tapering end (Pl. XII, Fig. 19). In cleistogamous flowers the generative cell may divide within the pollen-grain, or in the pollen-tube after germination has begun. The male gametes, with their pointed ends adjacent, are always preceded by the tube nucleus during the growth of the pollen-tube (Pl. XII, Figs. 20, 21). Parts of pollen tubes containing the male gametes and tube nucleus were seen in the stylar canal, and also within the ovary chamber. These preparations show that each male nucleus is still within a cytoplasmic sheath when the pollen-tube is about to enter the micropyle of an ovule. In many angiosperms, male cells, not merely male nuclei, are formed at spermatogenesis, and among these *V. Riviniana* may now be included. It is not yet known whether the whole male cell takes part in fertilization, but some

evidence for this is found in *V. Riviniana*. The later development of the pollen-tube is described in the account of fertilization.

Degeneration of the pollen. A considerable amount of the pollen degenerates in both cleistogamous and chasmogamous flowers. In the former degeneration may set in at almost any stage of development whether in the uninucleate or binucleate pollen-grains, or even after germination has begun.

CYTOLOGY OF THE EMBRYO-SAC.

A study was made of the early development of the embryo-sac from the megaspore mother-cell, both for its intrinsic interest and for comparison with the development of the pollen mother-cell. Comparisons of these critical stages in the development of the male and female gametophytes in any one plant are surely of great interest and of value to both cytologist and geneticist, but so far the attention of investigators has been focused more particularly upon the pollen development. The present comparison in *Viola Riviniana* cannot claim to be exhaustive, yet it brings out some striking points of similarity in the development of the pollen and megaspore mother-cells.

Resting megaspore mother-cell. The single archesporial cell, differentiated in the third layer of cells of the nucellus becomes the megaspore mother-cell direct, and is recognized by its size and comparatively dense granular protoplasm. The large spherical nucleus, containing a large nucleolus and scanty reticulum is very like the nucleus of a resting pollen mother-cell.

Meiosis. Heterotype division. During the first contraction phase, or synizesis, the reticulum becomes collected into a tight knot on one side of the nucleus: no continuous thread is distinguishable within it. The nucleolus is flattened against the nuclear membrane, either close to the knot, or on the opposite side of the nucleus. Some variation in the form of the nucleolus occurs, but not to such an extent as is noticeable in the pollen mother-cells. Careful examination of the nuclei at this stage shows that the knot is connected to the nucleolus by a fine thread or threads. The deep stain often taken by that part of the nucleolus to which the thread passes suggests that it may have a function similar to that suggested for the nucleolar body in the pollen mother-cell, although no well-defined nucleolar body is seen (Pl. XI, Figs. 13, 14).

In early diakinesis the twenty bivalent chromosomes are connected to one another by linin threads; these connexions later break down, and the newly-formed bivalents are somewhat spindle-shaped (Pl. XI, Fig. 15). The tapering ends are unstainable, and probably of linin material, while the central part is quite distinct and stainable. It seems just possible, from a comparison with the process of spindle formation described by Hughes-

Schrader (12) in the first maturation division of the egg of *Acroschismus* that these unstainable extensions of the bivalent chromosomes may be incipient spindle fibres. The bipolar heterotype spindle would then be formed by a later orientation and amalgamation of the twenty small spindles. However, these continuations certainly seem to disappear during diakinesis, and since spindle formation was not studied in detail, this is left as a suggestion only. The bivalent chromosomes are all about equal in size and more or less diamond-shaped. Linking of the chromosomes occurs in some megaspore mother-cells at this stage, but it does not seem to be a constant feature.

These, then, are the most important features in meiosis, where there is striking resemblance between the development of the megaspore mother-cell and the pollen mother-cell:

1. The connexion of the spireme to the nucleolus during the early prophases.
2. The variation of nucleolar form during first contraction.
3. The form of the bivalent chromosomes during early diakinesis, showing the unstainable continuations.
4. The linking of the bivalent chromosomes to one another during the heterotype division.

Synergidae. The chief interest in the eight-nucleate embryo-sac centres round the synergidae, and, in particular, their filiform apparatus (Pl. XII, Figs. 22-26). The synergidae are very similar in form to those described by Ishikawa (13) in the embryo-sac of *Oenothera*. Each is a large pyriform cell tapering towards the micropylar end of the sac. A distinct and well-developed filiform apparatus forms an apical cap in each of the synergidae; the base of the cell is occupied by a large vacuole, and the nucleus is found in the cytoplasm above this vacuole. The visor-like notch appears as a lateral indentation in the cytoplasm.

The Filiform Apparatus. The presence of a highly refractive cap at the vertex of the synergidae, giving a fibrillar appearance under the microscope, was recorded as early as 1856 by Schacht in his observations on *Gladiolus segetum*. This specialized part of the cell he described as a bundle of threads running longitudinally over the exterior of the vertex of the synergid, and he called it the filiform apparatus. It is a structure confined to angiosperms, and is recorded in the embryo-sacs of many monocotyledons and dicotyledons. Although little is known about it, considerable advance in the study of the filiform apparatus was made by Strasburger, and later, by Habermann (11). These workers found that it is a cap of cellulose nature, pierced by canals, in the apical cytoplasm of the synergid. Habermann observed that sugars and possibly proteins are present in the synergid vacuole, and suggested that these substances are secreted by the filiform apparatus into the micropyle, where they provide a chemical stimulus

for the pollen-tube. In his account of the synergidae of *Oenothera*, Ishikawa (13) states that the filiform apparatus is a solid mass of conical shape perforated by a number of minute canals which converge from the base to the apex.

The synergidae in the embryo sac of *V. Riviniana* seem particularly favourable for a more detailed study of the filiform apparatus, which is equally well developed in the embryo-sacs of cleistogamous and of chasmogamous flowers. As a result of this study some evidence is brought forward which suggests that the filiform apparatus is composed of a plexus of minute pores or tubules in a stainable matrix rather than merely canals in the protoplasm.

The early development of the filiform apparatus was not followed, but since the apical protoplasm of the young synergidae contains numerous minute round vacuoles, having almost a honeycomb appearance, it seems that the filiform apparatus is vacuolar in origin. In fully formed synergidae the filiform apparatus is stained by orange G, congo red and lichtgrün, but these transparent stains are of no use in elucidating the structural details. With Clausen's iodine gentian violet stain the filiform apparatus appears as a bright purple cap in each synergid, and presents quite a striking appearance under magnification. The use of this stain made it possible to study its structure in some detail. Considerable variation is observed in the appearance of the filiform apparatus in different preparations. Usually it appears to be a solid cap, stained purple, perforated by numerous small unstained pores wider at the base, and converging towards the apex: sections in different planes show the same structure (Pl. XII, Fig. 22). Quite frequently the filiform apparatus is seen as a rather ragged cap from the base of which long irregular prolongations extend into the cytoplasm. This appearance may be due to faulty fixation or to degeneration (Pl. XII, Fig. 23). In one preparation of the ovary of a cleistogamous flower the pores of the filiform apparatus appear as well-defined tubules, each one conical in form, and wider at the open basal end. These are much larger than the pores described above, and much fewer in number. The walls of these tubules are stained and the contents unstained. Since only one, out of the numerous preparations examined, shows this structure of the filiform apparatus, it may be a special case (Pl. XII, Fig. 24). Although chlor-zinc-iodine stains it blue, and it is dissolved by zinc chloride in hydrochloric acid, when treated with freshly prepared cuprammonia for twenty-four to forty-eight hours, or even longer, the filiform apparatus was never dissolved: it either remained apparently unchanged or else became altered in some way into a mass of minute stainable rods of a granular appearance, each of which, from their number and size, probably represented one of the original pores (Pl. XII, Fig. 25). This effect was not observed frequently. Thus, although the first two tests suggest that the filiform apparatus is composed

of cellulose, the cuprammonia reaction certainly seems to throw some doubt upon this conclusion. The filiform apparatus is a very special part of the synergid: this is obvious from the part it plays during the entry of the pollen-tube, described later. The suggestion made by Habermann that substances are secreted through the filiform apparatus into the micropyle is supported by this investigation. Specially stainable granular and globular bodies often occur in the synergid cytoplasm; these may represent the substances secreted (Pl. XII, Fig. 26). It would seem that the tubular structure suggested in this paper would bring the form and function of the filiform apparatus well into line.

Occurrence of more than one embryo-sac. The development of more than one megaspore of the tetrad was observed in the ovules of some cleistogamous flowers fixed in the spring of 1927. In every case one functional eight-nucleate sac had developed from the chalazal megaspore. In some ovules one extra megaspore had reached the binucleate or the tetranucleate stage, and there were three instances in which two extra megaspores had developed as far as the tetranucleate stage.

FERTILIZATION.

Entry of the pollen-tube. When the pollen-tube reaches the end of the stylar canal it either grows down against the inner surface of the ovary wall or grows out into and traverses the ovary chamber. The entry of the pollen-tube into the embryo-sac is similar to that described for *V. odorata*. In *V. Riviniana* the pollen-tube grows right through the filiform apparatus of one synergid, there becoming very much constricted, and it seems to continue for some distance into the synergid cell, but does not apparently grow right through it. The contents of the synergid are disorganized by this invasion, and appear merely as a deeply stainable mass. The other synergid remains intact until after fertilization.

In the case of some cleistogamous flowers whose embryo-sacs had not been entered by a pollen-tube, the nucleus in one synergid had degenerated, while that of the other synergid was quite normal. This degeneration is interesting in view of the part played by one synergid in the reception of the pollen-tube. It suggests that there may be a physiological difference between the two synergids: that the pollen-tube enters a particular one, and if no pollen-tube comes, the degeneration nevertheless occurs as a matter of course.

Sexual and triple fusion. It is probable that the end of the pollen-tube dissolves, and the contents flow out through the base of the synergid into the embryo-sac. Much deeply stainable material is carried into the embryo-sac, and this surrounds the ovum; it disappears quite soon. In spite of repeated efforts to demonstrate the first stages in fertilization, the earliest stage observed was that at which nuclear fusion had already begun. The

passage of the male gametes within the embryo-sac must therefore be very rapid. No changes in the form of the male nuclei after liberation from the pollen-tube such as those described for *V. odorata* were observed in this species. The male nuclei in the embryo-sac do not contain any stainable reticulum, and are apparently in a resting condition. They are quite different in structure from the reticulate male nuclei observed in the pollen-tube within the ovary, but it is not known when the change takes place. The first male nucleus is found flattened against the ovum nucleus on the side opposite to the destroyed synergid. The larger nucleolus of the ovum and the nucleolus of the male nucleus remain distinct for some time after the nuclear membranes have fused. The polar nuclei are still separate, and the second male nucleus fuses with one of these. This male nucleus is represented almost entirely by its nucleolus, which is at first distinct from the nucleolus of the polar nucleus, but later forms one large oval fusion nucleolus with it. It was not observed whether a single definitive endosperm nucleus is formed.

The above account rather suggests that the male gametes are represented only by their nuclei at fertilization, and so it is of interest to note that in a few cases a small stainable body appears close to the first male nucleus at sexual fusion. This body recalls the stainable 'dot' in the 'tail' of the male cell, and although the cytoplasmic sheath was not seen within the embryo-sac, it suggests that the first male nucleus is accompanied by some of the cytoplasm, and there is also the possibility that this takes part in the fusion.

A cellulose membrane is secreted around the fertilized egg, which does not divide until after four or eight free endosperm nuclei are formed. The later development within the embryo-sac shows no particular points of interest.

DISCUSSION.

The bivalent chromosomes. The linking between the bivalent chromosomes during the meiotic divisions in *V. Riviniana* is a feature not previously recorded in plant cytology, and a consideration of the possibilities offered by this linking, though necessarily tentative, may not be without value. The only reference, of which the writer is aware, to such a linking of bivalent chromosomes, is made by Clausen (2) in describing an illustration of a heterotype metaphase figure in the pollen mother-cells of *V. arvensis*. The chromosomes are scattered over the spindle and are connected by threads, and Clausen says that 'the chromosomes seem to have difficulty in separating'. In a later paper (4) he states that the twenty bivalent chromosomes of *V. Riviniana* lie far apart at heterotype metaphase and are easily counted. The formation of groups of linked chromosomes during the heterotype division is recorded for *Oenothera* spp. by Cleland (5, 6) and by Sheffield (19). In these species of *Oenothera*, however, there is no true

diakinesis stage during which pairing of homologous maternal and paternal chromosomes takes place. The chromosomes formed from the univalent spireme which emerges from synapsis do not all form pairs, but all or some are linked by fine threads in a chain or a ring of fourteen or of a smaller number. The arrangement of the chromosomes seems to be constant for any one species. The chromosomes are still linked at heterotype metaphase, and in anaphase adjacent chromosomes pass to different poles and thus the separation of the homologous univalents is brought about. In *V. Riviniana*, however, the linking is of the bivalent chromosomes and does not replace diakinesis, so that it is essentially different from the linking in *Oenothera*. Three possible explanations suggest themselves. Firstly, the occurrence of linking in early diakinesis suggests that the chromosomes are not separated in the last premeiotic resting nucleus: that they, or their representatives are already arranged in a certain order in the spireme and that by this linking the particular arrangement is maintained throughout meiosis. The linking in *Oenothera* shows that the univalent chromosomes are already in order in the post-synaptic spireme. Secondly, if the bivalent chromosomes are linked together as soon as they are formed, or possibly before, and the linking persists throughout the meiotic division, the independent assortment of the chromosomes, giving all the possible combinations in the pollen-grains and megaspores, is necessarily very much limited. Such an effect of the linking would be comparable with, but probably much more comprehensive than the linking of the genes, since presumably, entire chromosomes pass linked together into the megaspores and microspores. Thirdly, in some cases the linking is so complete as to amount almost to a fusion between some of the bivalent chromosomes, and here there certainly seems to be an opportunity for interchange of material, possibly genetical, between the ends of adjacent chromosomes. A process analogous to 'crossing over' between homologous chromosomes is suggested here. In view of all the evidence now supporting the individuality of the chromosomes, and in particular the construction of chromosome 'maps', this suggestion is a little bold; nevertheless, the possibility exists. It must always be remembered that in *V. Riviniana* much variability is seen in the degree of this linking, and this would seem to impose serious limitations upon any theoretical application which it suggests. Further evidence for the existence of this linking phase would be welcome, and it would be of considerable interest to investigate other species of *Viola* with this end in view.

The male gametes. The male nuclei in *V. Riviniana* never become vermiform, and there is therefore nothing to suggest that they are capable of movement. At the same time, the form of the whole male cell certainly indicates motility and the appearance of a small deeply stainable body at one pole of the resting generative nucleus suggests, superficially at least, a

comparison with the blepharoplast of antherozoids. A similar deeply staining granule is described in the generative cell of *Lilium auratum*, and it is suggested by Welsford (20) that this granule may be a centrosome, but no spindle fibres were seen near it. Other granules occur in the cytoplasm of the generative cell of *Lilium auratum* between the nucleus and the wall. These are extruded from the nucleus, sometimes forming a band-like structure, and are considered to be vestiges of a blepharoplast. The later history of the body in the generative cell of *V. Riviniana* is not known. So far it has not been seen at the pole of the spindle, so that it may not function as a centrosome. Nevertheless, it is probable that this body fragments and gives rise to the granules which appear on the spindle at telophase and are included in the cytoplasm. The presence of a deeply stainable body near the first male nucleus at sexual fusion is noted in the account of fertilization.

Crystal bodies in the nucleolus. The presence of crystal bodies in the nucleolus is recorded in different parts of plants and at various stages of development. For instance, crystalline structures are present in the nuclei of the root tip-cells of *Galtonia candicans* (8); these are not within the nucleolus, but are thought to originate from this. In *Allium cepa* (18) regularly shaped, highly refractive bodies occur in the resting phase nucleoli: they are apparently absorbed during spireme formation. The nucleoli of the resting nuclei of *Anacyclus Pyrethrum* (17) frequently contain a refractive body, crystalline in structure. Similar crystals are present regularly in the premeiotic pollen mother and tapetal cells of *Lathyrus odoratus* (19), and with varying frequency in the pollen mother-cells of *Oenothera* spp. (13), of *Lathraea clandestina* and *L. squamaria* (10), and are recorded in the endosperm nuclei of *Macrozamia Fraseri* before free nuclear division (15). To these instances the premeiotic pollen mother-cells, tapetal-cells, and nuclei of the developing and mature embryo-sac of *V. Riviniana* may now be added, although the crystals are not always observed. It is suggested by Gates and Latter (10) that the constant occurrence of these crystals in cells entering upon an increased period of activity indicates that they are associated in some way with the metabolism of the cells. This is supported by the fact that crystals occur in those cells of *V. Riviniana* which are entering upon either of the two cytological crises in the life history of the plant, viz., reduction division and nuclear fusion.

The nucleolar body. The close association of the spireme with the nucleolus during the meiotic prophase in which the spireme is formed is now recognized in several plants, and the conclusion is very naturally drawn that the nucleolus holds a source of chromatin material which is supplied to the spireme. The presence of a special nucleolar body with which the connexion is maintained was first described by Latter in the pollen mother-cells of *L. odoratus*, who suggests that this body elaborates chromatin

from prochromatin material within the nucleolus and then transfers the chromatin to the spireme. The term 'nucleolar body' is adopted in the present account of the pollen mother- and megaspore mother-cells of *V. Riviniana* since, from its position, staining properties and apparent function, it is quite similar to that in *Lathyrus*. The origin of this body in *V. Riviniana* is, however, unknown. Since crystal bodies are not frequently seen in the resting pollen mother-cell nucleoli it seems doubtful whether it originates from one of these, as it probably does in *Lathyrus*.

SUMMARY.

Morphology.

1. The general morphological features of the chasmogamous, semi-cleistogamous, and cleistogamous flowers of *Viola Riviniana* are described, and the annual cycle of flower production is given.

2. The semi-cleistogamous flowers seem to show, in a series of forms, a gradual reduction from chasmogamy to cleistogamy. These flowers exhibit the following features: (a) reduction of petals and closure of flower, (b) reduction of pollen-sacs, (c) germination of the pollen within the anther, and (d) the curvature of the style over towards the anterior stamens.

3. The cleistogamous flowers show extreme reduction of pollen-sacs, and the style is curved so that the stigma is almost in contact with the anterior stamen sacs.

4. Two kinds of pollen-grain are found in the anthers of chasmogamous flowers: many large grains, which are shed, and a few smaller grains which germinate within the anther. The latter are similar in size and form to the grains of cleistogamous flowers.

5. The chasmogamous flowers often fail to set seed; the cleistogamous flowers set good seed, except perhaps towards the end of the season.

Cytology.

6. The haploid number of chromosomes of *V. Riviniana* is 20.

7. Pollen meiosis. During first contraction, open spireme and second contraction, the spireme is connected to the nucleolus by a thread or threads, passing to the nucleolar body.

8. In early diakinesis and throughout the later stages of the meiotic division the bivalent chromosomes are linked into groups.

9. Pollen tetrad formation takes place by the grooving of the pollen mother-cell cytoplasm and accompanying ingrowth of wall material from the thick pollen mother-cell wall. This disappears later, and a wall is secreted around each pollen-grain.

10. Two male cells are formed at spermatogenesis. Each contains a spherical reticulate nucleus and one or two chromatin bodies in the cytoplasm of the 'tail'.

11. Crystal bodies frequently occur in the nucleoli of the newly differentiated, uninucleate tapetal cells. During pollen development the tapetal cells become multinucleate.

12. Degeneration of the pollen in cleistogamous flowers may set in at any stage of development.

13. The eight-nucleate embryo-sac is normal in development and in construction. The synergids have a well-developed filiform apparatus. The polars retain their individuality until after fertilization.

14. The pollen-tube enters one synergid through the filiform apparatus. The two male gametes and some stainable material from the pollen-tube are liberated through the base of the synergid into the embryo-sac.

15. The second male nucleus fuses with one polar nucleus, and this fusion nucleus remains distinct from the second polar nucleus for some time.

16. Cases of the development of extra immature embryo-sacs in some ovules are recorded.

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DESCRIPTION OF PLATES XI AND XII.

Illustrating Miss G. West's paper on Cleistogamy in *V. Riviniana*, with special reference to its Cytological Aspects.

All figures drawn with camera lucida and reproduced without reduction. Obj. Zeiss apo. 2 mm oil imm. N.A. 1.3. Ocular Zeiss K10, K12, K18. Magnification of figures: Figures 1-8, 13-15. $\times 3,300$ (Oc. K18). Figures 9-12, 16-21. $\times 2,100$ (Oc. K12). Figures 22-32. $\times 1,350$ (Oc. K10).

Abbreviations used: (1) Fixatives. A.B. Allen's modification of Bouin's fluid. F.W. Flemming's fluid weak. F.S. Flemming's fluid strong. A.A. Acetic Alcohol. M.C. Medium-Chrom-Acetic. (2) Stains. H. Heidenhain's iron-alum haematoxylin. I.G.V. Clausen's modification of Gram's iodine gentian violet method. G.V. Newton's modification of Gram's method.

PLATE XI.

Figs. 1-8. Pollen development. Meiosis.

Fig. 1. A resting pollen mother-cell; the nucleolus contains a small crystal body and a small vacuole; the reticulum is very slight. A.B.; I.G.V.

Fig. 2. First contraction (synthesis). The contraction knot is connected to the nucleolus by delicate threads; the nucleolar body appears as a small deeply stainable projection on the surface of the nucleolus. A.B.; H.

Fig. 3. Open spireme. The spireme is scattered throughout the nucleus, and is connected by one, or possibly more, delicate threads to the nucleolar body. F.W.; G.V.

Fig. 4. Part only of a nucleus showing an early stage in the formation of bivalent chromosomes. The paired chromosome initials appear as small stainable areas in a rather thick granular thread. The spireme is much attenuated, except where chromosome formation is taking place, and the connecting threads are scarcely seen. F.W.; G.V.

Fig. 5. Early diakinesis. The univalent halves of the twenty bivalent chromosomes are quite distinct; faint linin continuations of the chromosomes are seen, and delicate threads apparently still connecting some of the bivalents. F.W.; G.V.

Fig. 6. Later diakinesis. Faint linin threads still appear between the bivalent chromosomes, which are somewhat larger than in Fig. 5. The nucleolus, much smaller than in Figs. 4 and 5, is beginning to disappear. F.W.; G.V.

Fig. 7. Late diakinesis. The nucleolus appears merely as a small faintly stainable area in the nucleus. Many of the bivalents are now compact, although the double nature is still seen in a few. The twenty chromosomes are seen, and some are already linked. F.W.; G.V.

Fig. 8. Intracellular spindle formation. The spindle fibres diverge from one point within the nucleus; the nuclear membrane is still present on the opposite side of the nucleus. A.B.; H.

Figs. 9-12. Pollen development. Pollen-grain wall formation.

Fig. 9. Beginning of grooving of cytoplasm and ingrowth of thick pollen mother cell-wall. The

spindle fibres have almost disappeared, and indications of evanescent cell-plates are seen in the cytoplasm. A.B.; H.

Fig. 10. The ingrowing walls have almost reached the centre. A.B.; H.

Fig. 11. Tetrad formation completed; each microspore has a delicate wall of its own; the thick tetrad wall is dissolving. A.B.; I.G.V.

Fig. 12. Three pollen-grains of a tetrad still connected by a few traces of the old pollen mother-cell wall. A.B.; I.G.V.

Figs. 13-15. Megaspore mother-cell. Meiosis.

Fig. 13. Early open spireme. The nucleolus has become spherical, and the spireme is apparently connected to it. Chromatin transference seems to be in progress, and the chromatin appears to have accumulated at some points on the thread. F.W.; G.V.

Fig. 14. The nucleolus of a megaspore mother-cell showing the connexion of threads to the deeply stained area which is probably of the same nature as the nucleolar body in the pollen mother-cell. M.C.; G.V.

Fig. 15. Diakinesis showing the twenty bivalent chromosomes. The halves of the chromosomes are very clear and show the unstainable continuations, which are probably of linin. A.B.; H.

PLATE XII.

Figs. 16-21. Spermatogenesis.

Fig. 16. Generative cell before division. The chromatin body is seen at one pole of the nucleus. A.B.; I.G.V.

Fig. 17. Metaphase, profile view, of division of generative nucleus. A.B.; I.G.V.

Fig. 18. Telophase of division of generative nucleus; the division figure occupies almost the whole length of the cell. The nuclei show a stainable reticulum, and a cell-plate is formed at the equator. The chromatin bodies, possibly formed by the fragmentation of the large one seen in Fig. 16, are seen upon the spindle. A.B.; I.G.V.

Fig. 19. Formation of the male cells by constriction of the protoplasm of the generative cell on either side of the cell-plate. The spindle fibres have disappeared; the bending of the cell shown is not usual; it may be due in this case to the limits imposed by the pollen-grain wall upon the elongation of the cell. The chromatin bodies are only faintly shown in this pollen-grain. A.B.; I.G.V.

Fig. 20. Part of a pollen-tube in the anther sac—showing the two male cells and the tube-nucleus. F.S.; H.

Fig. 21. Part of a pollen-tube in the ovary, showing the two male cells and the tube-nucleus. This preparation is much de-stained, which may account for the apparent absence of chromatin bodies. F.W.; H.

Figs. 22-6. Synergidae. Filiform apparatus.

Fig. 22. Superficial view of the vertices of the synergids in tangential longitudinal section, showing the well developed filiform apparatus. The ends of the pores or canals appear as small clear areas in the stained matrix. A.B.; I.G.V.

Fig. 23. Filiform apparatus showing stringy prolongations extending into the synergid cytoplasm. F.W.; I.G.V.

Fig. 24. Longitudinal section of the filiform apparatus. The preparation suggests that the apparatus is composed of definite tubules. A.B.; I.G.V.

Fig. 25. Filiform apparatus after treatment with cuprammonia, and subsequently stained; it seems to have been changed into a mass of rods which are stainable. A superficial view is drawn, the filiform apparatus of one synergid only appears in the section. A.B.; I.G.V.

Fig. 26. Synergids showing cytoplasmic inclusions, some of which are of a highly refractive nature. F.W.; H.

Figs. 27-32. Fertilization.

Fig. 27. Composite drawing from sections through one embryo-sac. The sac has been entered by a pollen-tube, and the destroyed synergid is drawn merely in outline; the other is quite healthy. Some deeply stained material from the pollen-tube surrounds the egg-cell. The first male nucleus appears near the egg-nucleus, and the second male nucleus is pressed against one polar nucleus. A small chromatin body is seen near the second male nucleus, and a more faintly stained one near the first male nucleus. A.B.; H.

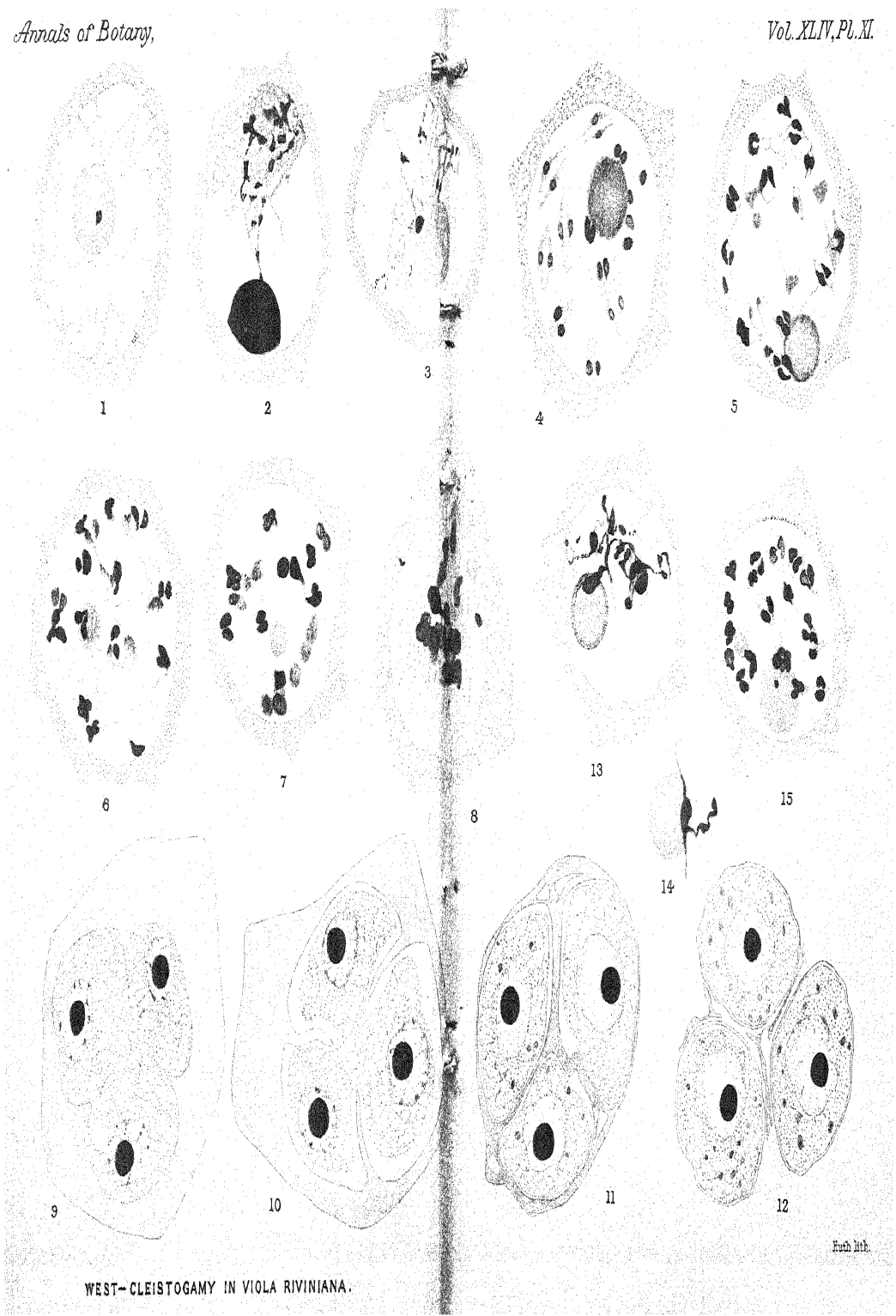
Fig. 28. An active male nucleus with a reticulum appears within the egg-cell: the nucleolus of the egg-nucleus contains a crystal. A.B.; I.G.V.

Fig. 29. Fusion of the first male and egg nuclei; the male is flattened against the female nucleus. The preparation shows much stainable material around the egg-cell. A.B.; H.

Fig. 30. After nuclear fusion; the male nucleolus is seen within the nuclear area of the egg-cell A.B.; H.

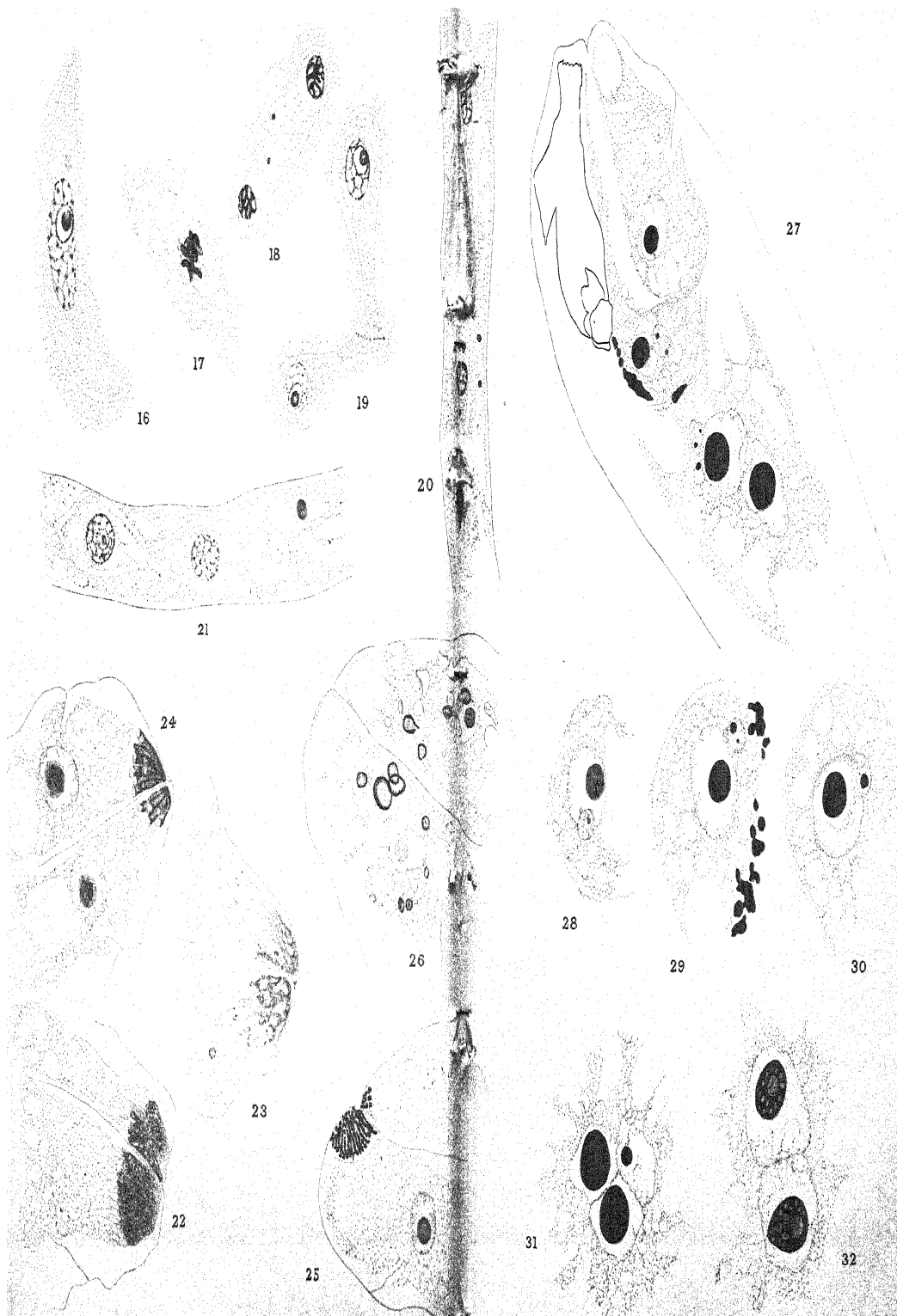
Fig. 31. The two polars and the nucleolus of the second male before fusion of nucleoli; the male nucleolus is within one polar nucleus. Figs. 29 and 31 are taken from sections through the same embryo-sac. A.B.; H.

Fig. 32. The lower larger nucleus is the one formed by the fusion of one polar and a male nucleus; the other polar nucleus is still distinct. The nucleoli are very vacuolate. A.A.; H.



WEST-CLEISTOGAMY IN VIOLA RIVINIANA.

Each 1/100.



The Distribution of Primulas from the Himalaya to China, with Descriptions of some New Species.

BY

F. KINGDON WARD.

With one Figure in the Text.

IN the years 1924-8 I made three expeditions into the tangled mountainous country lying between Sikkim in the west and Yunnan in the east, between the parallels of 28° N. and 30° N. These expeditions were financed by the Percy Sladen Memorial Fund and the Government Grant Committee of the Royal Society, and received also private support. The object in view can be stated in a few words.

It has long been recognized that there is a general resemblance between the flora of the Himalaya and that of western China. Hooker, Watt, C. B. Clarke and others have drawn attention to this fundamental relationship. Drude, in his distribution maps, has depicted the East Asiatic region as extending unbroken along the Himalayan ranges.

On the other hand a legend had grown up that the flora of Sikkim at any rate was unique, that is to say, largely endemic and possibly the richest, for the size of the valley, in eastern Asia. Latterly a very similar belief has grown up with regard to the flora of western Yunnan.

Before the war it had occurred to the writer to investigate more closely this general relationship, in the hope of discerning the more special relationship which must exist; thereby throwing light on the biological history, and checking, and perhaps supplementing, the geological history of one of the most fascinating regions in the world. For we have to deal with the meeting-place of three distinct floral regions—the Central Asiatic, Indo-Malayan, and East Asiatic. Nor is that all. These regions are cut off from one another, not by seas, but by ranges of mountains, which are in the nature of things, not only barriers, but also carriers.

Thus it was hoped to obtain, in particular, more exact information as to the limits of distribution of genera and species, without which it would be impossible to understand either their migrations in the past or their distribution in the present. This information might be more valuable to the plant geographer than the discovery of new species.

Bearing the above objects¹ in view, the following expeditions were planned; it being assumed that the floras of Sikkim-Bhutan in the west, and of Yunnan-Szechuan in the east, at either end of the great gap between the Yang-tze and the Brahmaputra, were, by that time, fairly well known.

(i) 1924-5. To the Tsangpo gorge in Tibet, and the Assam Himalaya. The main botanical collections came from the eastern Himalaya, on both sides of the Tsangpo: lat. 30° N., long. 95° E.

(ii) 1926. To the headwaters of the Irrawaddy, Burma-Tibet frontier. Main botanical collections from the Irrawaddy-Brahmaputra divide, Seinghku valley: lat. $28^{\circ} 10'$ N., long. $97^{\circ} 25'$ E.

(iii) 1928. To the Mishmi Hills, Assam-Tibet frontier. Main botanical collections from the Delei valley, Lohit river: lat. $28^{\circ} 15'$ N., long. $96^{\circ} 30'$ E.

Although as a result of these journeys, unexpected light has been thrown on the distribution of genera and species between Yunnan and Sikkim, it is not yet possible to draw any definite conclusions regarding the past history of this region. No such attempt is made here. Nevertheless several interesting facts have come to light, and may be illustrated by reference to certain genera, the genus *Primula* being here selected for that purpose.

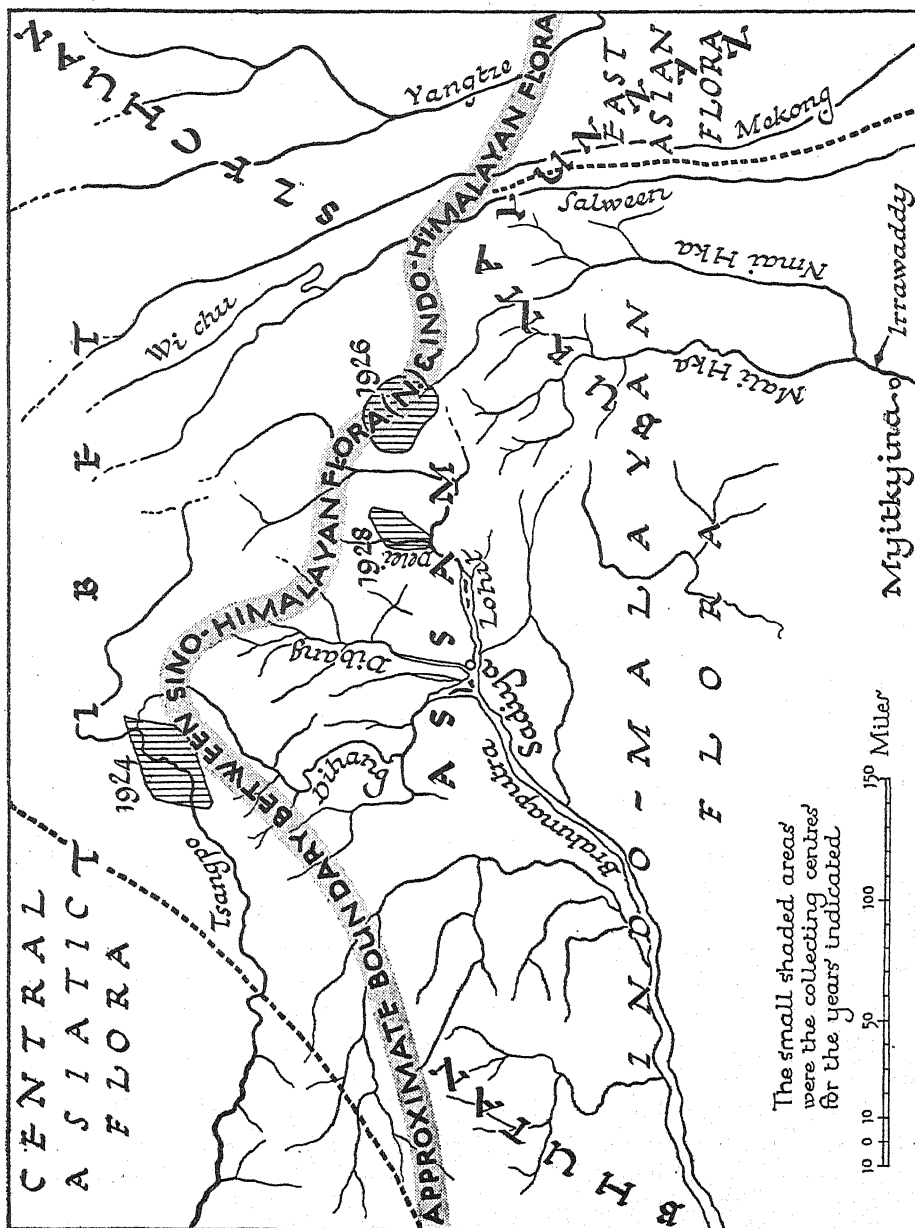
Reversing the usual procedure, I have summarized the main conclusions arrived at before passing on to their illustration. These conclusions are as follows:

(i) The Sikkim flora is not confined to Sikkim, but extends eastwards across the mountains of Assam and Burma to Yunnan. The obvious corollary, that the Yunnan flora extends westwards to the Himalaya is equally true.

(ii) The floras, alpine and sub-alpine, of the zone of contact between the three floral regions enumerated above, have an east to west extension irrespective of the general strike of the country. While we should expect this flora to extend east and west along the ranges which lie to the west of the Tsangpo bend, we should equally expect it to extend north and south along the ranges which lie to the east of Assam, since the strike of the country is there north and south. The fact remains that it does not; both alpine and sub-alpine floras continue to follow an approximately east and west alignment.

(iii) The alpine flora is divisible into two main belts which have developed separately, one on either flank of the Great Himalayan range and its eastward prolongation. The northern dry climate flora may be called Sino-Himalayan, the southern wet climate flora Indo-Himalayan. Neither belt has any appreciable north to south extension, although in the east the belts do tend to broaden, following the strike of the country.

¹ The introduction of new plants of horticultural, scientific, or economic value was also an object.



(iv) The Sino-Himalayan flora, in the east, tends to follow a north-easterly course; plants of the N.W. Himalaya and of the Tsangpo valley tend to reappear in Kansu and Szechuan. The Indo-Himalayan flora, in the east, tends to follow a southerly or south-easterly course; and plants of the eastern Himalaya tend to reappear in southern Yunnan and in Upper Burma, even in the Naga Hills. The strike of the country is mainly responsible for this.

(v) These two floras have developed in isolation on either side of the main range, separated from one another by the ice-sheet which covered the Himalaya during the last glacial epoch. This ice-sheet allowed them to spread east and west, but in no other direction. Since the long-drawn-out retreat of the ice in modern times, the barrier between them has been removed, and many species have crossed the passes in either direction. Yet the two floras remain on the whole remarkably distinct.

It has already been mentioned that the country under consideration is the meeting-ground of three floral regions. To the north of the Great Himalayan range lies the plateau of Tibet, with its semi-desert xerophytic flora of Central Asiatic affinity. To the south and south-east of the Himalaya lie the warm monsoon regions of India, Burma, and Indo-China, forming, with the islands of the East Indian Archipelago, the Indo-Malayan floral region. Finally, to the east of the Burma-Yunnan ranges, and stretching away to the north-east, is the Chinese flora proper covering the bulk of the East Asiatic region. Throughout the zone of contact of these three floral regions, the country is pervaded by great ranges of mountains, which belong to two different geological epochs, and whose strikes are in conflicting directions. These ranges act in a dual capacity; on the one hand they form barriers between adjacent regions. With regard to (i) above, that the Sikkim flora extends eastwards and the Yunnan flora westwards, it will be sufficient to mention the following facts in support of the statement:

Sikkim plants recently found to extend eastwards:

Meconopsis Wallichii, to Burma (1914) and Szechuan; *M. paniculata*, to Assam (1928); *M. lyrata*, to Burma and Yunnan; *Primula Wattii*, to Burma (1926); *P. prenantha*, to Assam (1928) and Burma (1926); *P. sikkimensis*, to Burma (1926); *P. muscoides*, to Burma (1926); *Rhododendron pumilum*, to Burma (1926) and Assam (1928); *R. virgatum*, to Assam (1928) and Burma (1926); *R. Nuttallii*, to Assam (1928) and Burma (1926); *R. Roylei*, to Assam (1928); *Nomocharis nana*, to Burma (1926); *Bryocarpum himalaicum*, to Assam (1928), *Magnolia Campbellii* and *M. globosa*, to Assam (1928) and Burma (1926).

Yunnan-Szechuan plants recently found to extend as far west as the Himalaya: *Primula Maximowiczii*, *P. szechuanica*, *P. chungensis*, *P. pulchelloides*, *P. congestifolia*, *P. vernicosa*, *P. sinopurpurea*; *Rhododendron repens*, *R. sinogrande*; *Magnolia rostrata*; *Rosa Moyesii*; *Meconopsis*

pseudointegrifolia; *Lonicera cyanocarpa*; *Gentiana Trailliana*; *Lactuca Souliei*; *Draba involucrata*; *Linaria yunnanensis*; *Clematis Forrestii*; *Pedicularis densispica*; *Adonis brevistyla*; *Allium yunnanense*; *Lilium hyacinthinum*; *Oxytropis yunnanensis*; and *Plectranthus orestius*; all found in the eastern Himalaya in 1924.

The horizontal or east-to-west extension of all these alpine and of the flora generally, greatly exceeds their meridional extension, even on the eastern ranges. No doubt the restricted meridional distribution, even of species confined to the Burma-Yunnan ranges, is partly due to the fact that the latter slope down to the south rather rapidly as they spread out fan-wise. But this is not the main cause.

The complete change in the type of vegetation noticed on crossing the Himalaya necessarily connotes a drastic alteration in the composition of the flora; in this sense therefore there are two floral belts, one on either side of the range. But the important point is that these two belts persist eastwards across the grain of the country; for a similar change in the type of vegetation may be observed when passing northwards through Yunnan, even though one is here travelling not *across*, but *parallel to* the main ranges. Indeed, on the Salween river, even at the bottom of the gorge, an *abrupt* change from Indo-Malayan jungle to temperate or Coniferous forest takes place in about lat. 28° N. Thus just as the flora of Sikkim-Bhutan can be traced eastwards across the head of the Assam valley, to the Burma-Yunnan ranges, so the flora of the Tsangpo valley can be traced eastwards across the headwaters of the Lohit and the Irrawaddy to Szechuan and Yunnan, north of the twenty-eighth parallel. The two lists of plants already given might with very few alterations be used also to illustrate the southern and northern belts respectively. *Nomocharis nana*, in the first list, properly belongs to the northern flora; *Primula vernicosa*, *Rhododendron repens* and *R. sinogrande*, in the second, belong to the southern flora. Otherwise the species named are representative of the southern or Indo-Himalayan, and northern or Sino-Himalayan floras respectively.

It is clear, however, that comparatively few *species* can be entirely confined to one side or the other. The main factor in their separation is climatic, and owing to various causes, the two climates are never sharply divided from one another. Again, in the high alpine region conditions are very uniform, and along the crests of the main ranges, the same species are usually met with for some little distance down either flank. Lastly, since the retreat of the ice-sheet, many species have crossed the passes in either direction, and invaded alien territory. It is to the *bulk* of the species of a genus, or in the larger genera, of a section, that one must look for confirmation; the genus *Primula* has here been selected, and the distribution of its sections analysed, for purposes of illustration.

It has been stated above that the cause of the separation of the flora

into two independent belts, which have only recently come into contact, were the conditions prevalent during the ice age.

At that time all the passes over the eastern Himalaya at any rate were blocked. The Doshong La and the Nam La, over the Assam Himalaya, which I crossed in 1924, were formerly occupied by glaciers. So also was the Diphuk La, crossed in 1926. Going farther east, the Doker La, Chu La and Pitu La, all of which cross the Mekong-Salween divide, with the Gompa La, which crosses the Salween-Irrawaddy divide, and the Paima La which crosses the Mekong-Yangtze divide, were formerly occupied by glaciers. North of the Tsangpo and east of the Mekong, the entire country seems to have been under ice, and there could have been no communication across the main ranges. Where glaciers still exist as in the Assam Himalaya, on the Salween-Irrawaddy divide, on the Ka-kar-po range of the Mekong-Salween divide, and on the Paima-shan range still farther east, all are in retreat. Their former extension is plainly indicated, especially in Tibet, where the much drier climate has preserved the form of the country; and it is certain that they have shrunk many miles in length and many hundreds of feet in thickness. In short, an arctic ice belt separated the plains of India from the plateaux of Tibet and Yunnan; and it is a reasonable inference that as the ice slowly retreated, the alpine flora, which had been driven down into the valleys on either side by the advancing ice, developed in isolation as they reascended in the wake of the ice. Nor did the river gorges in the east afford a readier means of communication between north and south; for at that time the gorges of the Mekong and Salween did not exist. These rivers then flowed at a much higher level than they do now, in wide plateau valleys; and the change in the character of the flora which is so noticeable as one ascends the gorges to-day must have been accentuated then (1).

Turning now to the genus *Primula*. Before plotting the distribution of *Primula* sections in this region, it will be convenient to say something about the collections made in 1926 and 1928. A list of all the species collected in Tibet in 1924, with comments and descriptions of new species, was published in the Notes, R. B. G., Edinburgh, February, 1926 (3); and descriptions of the seven new species collected in Burma in 1926 were published in the Notes, R. B. G., Edinburgh, October, 1927 (4). Below I have given a complete list of the species collected in 1926 and 1928 on the Burma and Assam frontier ranges (area $28^{\circ} 0' - 28^{\circ} 30' N.$ lat. $96^{\circ} 30' - 97^{\circ} 30' E.$ long.). New species are printed in italics.

Burma Frontier, 1926.

- K.W. 6820. *P. Agleniana*, Balf. f. et Forrest, var. *thearosa*, Ward., var. nov.
 „ 6822. *P. eucyclia*, W. W. Sm. et Forrest (also K.W. 7483).
 „ 6830. *P. blandula*, W. W. Sm. (also K.W. 6911, 7016, 7055).

- K.W. 6841. *P. euosma*, Craib. (also K.W. 6899).
 „ 6842. *P. serratifolia*, Franch. (also K.W. 7019).
 „ 6875. *P. prenantha*, Balf. f. et W. W. Sm. (also K.W. 6982, 7499).
 „ 6897. *P. calthifolia*, W. W. Sm.
 „ 6901. *P. melanodonta*, W. W. Sm. (also K.W. 7042, 7536).
 „ 6902. *P. sikkimensis*, Hook. (also K.W. 7041, 7054).
 „ 5904. *P. involucrata*, Wall., var. *yargongensis*, Petitm. (= *P. Wardii*, Balf. f. of gardens).
 „ 6910. *P. Genestierana*, Handt. Mzt. (also K.W. 7005, 7579).
 „ 6928. *P. chamaethauma*, W. W. Sm.
 „ 6929. *P. bryophila*, Balf. f. et Farrer (also K.W. 7021).
 „ 6933. *P. vernicosa*, Ward.
 „ 6940. *P. silaensis*, Petitm. (also K.W. 7002, 7590).
 „ 6957. *P. apoclita*, Balf. f. et Forrest (also K.W. 6975).
 „ 6981. *P. cyanantha*, Balf. f. et Forrest.
 „ 6986. *P. muscoides*, Hook. f.
 „ 6987. *P. bella*, Franch. forma.
 „ 7009. *P. Wattii*, King (also K.W. 7017).
 „ 7015. *P. crispata*, Balf. f. et W. W. Sm. (also K.W. 7175, 7226, 7507).
 „ 7018. *P. rhodochroa*, W. W. Sm. (also K.W. 7053).
 „ 7020. *P. fea*, Ward.
 „ 7040. *P. siphonantha*, W. W. Sm. (also K.W. 7228, 7551).
 „ 7052. *P. euchaetes*, W. W. Sm.
 „ 7080. *P. cyclophylla*, Balf. f. et Farrer.
 „ 7097. *P. sinopurpurea*, Balf. f.
 „ 7107. *P. chungensis*, Balf. f. et Ward.
 „ 7132. *P. alta*? Balf. f. et Forrest (in fruit).
 „ 7133. *Primula* sp. § *Farinosae*? (in fruit).
 „ 7227. *P. firmipes*, Balf. f. et Forrest (also K.W. 7512).

Assam Frontier, 1928.

- K.W. 8188. *P. alta*, Balf. f. et Forrest (also K.W. 8233).
 „ 8232. *P. vernicosa*, Ward (also K.W. 8240, 8408).
 „ 8235. *P. Clutterbuckii*, Ward (also K.W. 8421).
 „ 8252. *P. apoclita*, Balf. f. et Forrest (also K.W. 8406).
 „ 8262. *P. prenantha*, Balf. f. et W. W. Sm. (also K.W. 8347).
 „ 8282. *P. mishmiensis*, Ward (also K.W. 8393).
 „ 8292. *P. calthifolia*, W. W. Sm.
 „ 8295. *P. Normaniana*, Ward (also K.W. 8573).
 „ 8346. *P. septemloba*, Franch., var. *minor*, Ward, var. nov.
 „ 8347. *P. polonensis*, Ward (also K.W. 8388).
 „ 8361. *P. Agleniana*, Balf. f. et Forrest, var. *atrocrocea*, Ward, var. nov. (also K.W. 8404).

K.W. 8380. *P. rubra*, Ward.

„ 8381. *P. deleiensis*, Ward.

„ 8401. *P. silaensis*, Petitm.

P. capitata, or *P. crispata*, was also seen, out of flower.

The collections of 1926 and 1928 form an interesting contrast. In 1926 I found altogether thirty species of *Primula*. Analysis shows that of these, eleven were seen on the Burmese (wet) side of the range *only*; eight were found on the Tibetan (dry) side, or along the crest of the range, where the climate is Tibetan, *only*; and not more than five species were common to both flanks at any distance from the summit. The large number of species met with may be ascribed to the fact that I was able to collect in two climatic regions, and to the great altitudes reached.

In Assam, in 1928, we found only fifteen species all told, but of these, only seven were common also to the Seinghku valley, although the collecting areas are barely sixty miles apart, the two ranges being separated by the gorge of the Lohit. *P. Agleniana*, which is common to both areas, is represented by a different variety at each station, and both these differ from the type. The remaining six 'common' species have a much wider distribution.

P. calthifolia, which was so rare in the Seinghku valley, was abundant in the Delei valley, and is the only new species common to both areas. In other words the new species of both areas are rather local.

The number of new species found in Assam is 47 per cent. of the total number of species collected, and this in a country reckoned to be poor in *Primulas*! Much of the country is still totally unexplored; so that he would be a bold man who would put a limit to the number of species still to be discovered! But the discovery of new species is only half the result. Not a whit less important is the light thrown on distribution in general by the extension of old species; and it is now possible to link up, along clearly defined lines, Himalayan groups with their representatives in China.

To begin with, we may note a considerable extension eastwards of four Himalayan species: *P. prenantha*, *P. Wattii*, *P. muscoides*, and *P. rhodochroa*. There is a similar extension westwards of four Chinese species: *P. bella*, *P. serratifolia*, *P. vernicosa*, and *P. silaensis*. I had previously noticed, as a result of my Tibetan journey in 1924, the westward extension of other Chinese species, viz.: *P. chungensis*, *P. Maximowiczii*, *P. szechuanica*, *P. pulchelloides*, and *P. congestifolia*.

To these examples may be added the discovery of *P. septemloba*, var. *minor*, linking the Chinese *P. septemloba* with the Himalayan *P. geraniifolia*; of *P. chungensis* about midway between the type locality in Yunnan (lat. 28° N. long. 100° E.), and the Tsangpo valley, Tibet (lat. 29°30' N. long. 94°30' E); of *P. sinopurpurea*, midway between Yunnan and Tibet

(in Sikkim it is replaced by the almost identical *P. macrophylla* = *P. purpurea*, Royle); and of *P. congestifolia*, also midway between its Chinese and Himalayan localities.

The point to emphasize is that the species and sections of *Primula* are spread along a definitely east to west line, even in the gap between the Himalaya and the ranges of Central China; although athwart this gap the older Burma-Yunnan ranges cut clean across the strike of the country on either side. Further, that there are two distinct floral belts, a 'wet' belt to the south, and a 'dry' belt to the north, the division between them being as sharp in the 'gap', where there is no visible east-to-west barrier to act as a rain screen, as it is in the Himalaya, which stretches in the required direction.

The following general analysis of the distribution of *Primulas*, by sections, between the Himalaya and China, is significant. The sections are taken in the order adopted by Wright Smith and Forrest in their recent revision (2) of Bayley Balfour's classification, omitting only those which have no representatives in this region.

- § *Amethystina*. Sikkim to Assam, Burma, and Yunnan. None north of the main Himalayan range west of the 95th meridian, or north of 28°30' in Yunnan or Burma. High alpiners.
- § *Bella*. Sikkim (*P. pusilla*) to Yunnan (*P. bella*). None in Tibet. Sub-species of *P. bella* are found along the Burma-Yunnan frontier, from about 28°30' N. as far south as 26°. The horizontal extension is about four times the meridional.
- § *Bullatae*. Tsarong (E. Tibet) to Szechuan and Yunnan. No species are known from west of the Salween. The vertical distribution along the Sino-Tibetan frontier, from about 29° to 27° is roughly equal to the horizontal distribution. Plants of moderate altitude, mostly found on limestone.
- § *Candelabra*. Sikkim to Assam, Burma, and western China, with outliers in Japan and Java. The distribution is wide, and there are a large number of species; but with the exception of *P. chungensis*, none are found in Tibet, and only one in west China north of 28°30'.
- § *Capitatae*. Sikkim to Assam, Burma, and western China, just crossing the passes into Tibet. There is only one species, with variations.
- § *Cortusoides*. The sub-section *Eu-Cortusoides* is developed in northern and north-eastern Asia, whence it spreads southwards through Kansu and Szechuan to Yunnan. No species are found in the Sino-Himalayan mountains west of the 98th meridian; the distribution is meridional rather than lateral.

Sub-section *Geranioides* is distributed from Sikkim to Assam, Burma, and Yunnan, with outliers in Japan. One species, *P. latisecta*,

reaches Tibet ; the remaining species are found south of the Himalaya, and, in Yunnan, south of $28^{\circ}30'$. The distribution is lateral.

- § Denticulata. This section extends along both flanks of the Himalaya to Assam, Burma, and Yunnan.
- § Dryadifolia. Sikkim to Burma, Yunnan, and Szechuan ; and southwards along the Burma-Yunnan frontier as far south as 26° . High alpine confined to the crests of the main ranges.
- § Farinosae. The distribution of this section is world-wide.
- § Malacoides. This section only just touches the area in question with its most northerly species—*P. effusa*. It extends as far south as 23° , and the distribution is on the whole meridional. Low altitude plants of cultivated places.
- § Malvacea. This section is also confined to a small area in western China.
- § Minutissimae. Sikkim to Bhutan, with one species in western China. High alpine confined to the crests of the main ranges. Distribution lateral.
- § Muscarioides. Sikkim and Bhutan, south of the main Himalayan range, to Assam, Burma, and western China south of $28^{\circ}30'$. One or two species just cross the passes over the eastern Himalaya, into Tibet proper.
- § Nivalis. As a whole, this large section is distributed almost throughout Asia, and into north America as well. Certain types are, however, concentrated along definite bands. There is a high alpine series, *P. melanops*, *P. minor*, &c. (cf. *Amethystina*, and *Dryadifolia*), confined to the crests of the main ranges, from the eastern Himalaya to western China; another series, *P. szechuanica*, *P. Maximowiczii*, &c., extends from Tibet into western China, north of 28° , and up into Kansu ; a third series (*P. falcifolia* and *P. Agleniana* with its varieties) extends from the eastern Himalaya to Assam, Burma, and Yunnan south of 28° . Most of the remaining species extend generally from Sikkim to Burma and western China.
- § Obconica. Most of the species are found in Assam, Burma, and western Yunnan, south of the 28th meridian. *P. Listeri* occurs in Sikkim, while *P. obconica* itself is confined to Central China. Thus the distribution is lateral.
- § Obtusifolia. This section is confined to the high Himalaya and to Tibet.
- § Petiolares. A large section, extending from Sikkim and Bhutan to

Assam, Burma, and western China. At the extreme eastern end of the Himalaya one species, *P. Roylei*, crosses the passes into Tibet; in western China one species occurs north of lat. $28^{\circ}30'$.

§ *Rotundifolia*. Along both sides of the Himalaya from Nepal eastwards. Plants of high altitudes.

§ *Sikkimensis*. Along both sides of the Himalaya, from Sikkim to Yunnan and Szechuan; most abundant to the south, being found in Assam and Burma.

§ *Soldanelloideae*. Sikkim to Burma and Yunnan. High alpine, chiefly confined to the south side, only *P. Cawdoriana* crossing the passes into Tibet. In Yunnan they are found south of the 28th parallel.

§ *Souliei*. Mostly confined to western China, north of 28° , with an outlier, *P. xanthopa*, in Bhutan.

Of the twenty-one sections (out of thirty-two) represented in this area, two only fail to fulfil the first condition—that of lateral extension; and of these, one (§ *Malvacea*) has practically no extension at all. Two sections—*Farniosae* and *Nivalis*—have an almost world-wide distribution in the northern hemisphere, so that there is nothing remarkable about their distribution in these mountains, although § *Nivalis* tends to segregate into smaller groups, each of which has a definite, if limited, lateral extension. The remaining seventeen sections illustrate the lateral as opposed to the meridional distribution to which attention has already been drawn; one sub-section of § *Cortusoides* is meridional, the other lateral.

It will be noticed also how frequently a section, or the bulk of it, is concentrated either north or south of the main Himalayan range, and, for those sections which extend from the Himalaya to China, north or south of the 28th parallel (roughly) in Yunnan. This is perhaps even truer of individual species which may have quite a considerable lateral, but no meridional extension. Thus the sections *Amethystina* and *Bella* lie entirely south of our line, while the sections *Capitatae*, *Muscarioides*, *Soldanelloideae*, and *Petiolares* lie south of it, except for a few individual species which at particular points have crossed the passes. Similarly the sections *Candelabra*, *Obconica*, and *Souliei* have the bulk of their species confined to their respective sides. High alpine species such as *Dryadifolia*, *Minutissimae*, *Obtusifolia*, and *Rotundifolia* sit on the fence; they belong to neither side. Only two sections, *Denticulata* and *Sikkimensis* are so accommodating as to have a foot in either camp, and be equally at home in both; but these are plants of moderate elevations.

It is not to be expected that a complete proof of the dual origin of the mountain flora can be furnished by reference to a single genus. All that one can hope to do is to illustrate the principle as a basis for working out the distribution of plants in this region; and it will be found that the

principle holds good for a number of genera such as *Rhododendron*, *Magnolia*, *Meconopsis*, and *Nomocharis*.¹

The bearing of these facts on the geological history of the region of course opens up wider issues.

In conclusion, it will be convenient here to describe the new species of *Primula* collected recently, which have not hitherto been published.

K.W. 6820. *Primula Agleniana*, Balf. f. et Forrest, var. *thearosa*, Ward.

A more dwarf plant than the type (6-9 in. high) with leaves much less lacinate, and generally shorter pedicels. The flowers are a rich rose pink in colour.

Upper Burma. Seinghku valley, 28° 10' N., 97° 35' E. 9,000-10,000 ft. On old moraines amongst herbaceous growth and scrub *Rhododendron*. Flowers in June.

K.W. 8235. *Primula Clutterbuckii*,² Ward (*Farinosae-Yunnanensis*).

A tufted perennial with creeping rootstock, concealed by the persistent leaves of previous years. Leaves shortly stalked, the blade passing gradually into the petiole; spatulate or narrow oval, the petiole 1-1.5 cm. long, blade 2 cm. long, 6-9 mm. wide, the upper two thirds sharply serrate, the upper surface with scattered meal, the lower surface completely covered with meal which persists on the dead leaves. Flowers solitary or paired, always with bract and bracteole which may be 12 mm. long. Peduncle 2.5 cm. long, pedicel 6-9 mm. Calyx about 1 cm. long, cut half way down, the lobes acute, ribbed. Bracts pedicel, and calyx mealy on the outside. Corolla large, with very long tube, about 2 cm.; limb flat 2-2.2 cm. across, deep purple, the lobes obcordate, 1 cm. long. The upper part of the tube mealy, just powdering a ring round the base of the lobes.

Assam Frontier, Mishmi Hills. Delei valley, 28° 15' N., 96° 30' E. 11,000-12,000 ft. In crevices of gneiss cliffs, in shade. Flowers May-June.

K.W. 8282. *Primula mishmiensis*, Ward (*Nivalis*).

A glabrous perennial, 20-5 cm. high. Stock bulbous, surrounded by the withered leaves of the previous year, and consisting of the closely imbricate scale leaves, the outermost brown, the inner ones white or pink, passing into the green fleshy strap-shaped leaves; the scale leaves densely coated with whitish meal. Mature leaves—at flowering stage—petiolate, narrow-oval, obtuse, margin rather evenly obtusely dentate, upper surface dark green, smooth and shining, lower surface densely mealy, with prominent midrib and primary veins which at once break up, the ultimate ramifications ending in the marginal teeth. Blade about 10 cm. long, 3 cm.

¹ A recent paper by R. D'O. Good (Linnean Society's Journal, June 1929) on *Cremanthodium* seems to bear out the same conclusions; and especially point (iv) in the summary above.

² Named in honour of my companion, Mr. H. M. Clutterbuck, who first discovered it.

wide. Scape 20–25 cm. high, ending in a loose few flowered umbel. Bracts acicular, base widened, clasping the pedicel, 8 mm. long, mealy inside. Pedicel 12–14 mm. long, mealy. Calyx 1 cm. long, cut $\frac{2}{3}$ of the way, the lobes oblong, suddenly acute, thickly coated inside with meal which overlaps the edges. Corolla bright buff yellow, the deep crater coated with white meal. Tube (including crater) 1.5 cm. long, limb 2.5 cm. across, the lobes rounded, notched. Seeds orange brown, much wrinkled.

Assam Frontier. Mishmi Hills. Delei valley, 28° 15' N., 96° 30' E. 11,000–12,000 ft. On rocks and open alpine slopes, amongst scrub *Rhododendron*. Flowers June.

K.W. 8295. *Primula Normaniana*,¹ Ward. (*Cortusoides*–*Geranioides*).

An almost glabrous perennial 15–20 cm. high. Rootstock thin, creeping. Petiole 10–12 cm. grooved, and expanded at the base into a broad wing. Blade circular in outline, palmately cut into seven segments which are again sharply toothed; the sinuses between the lobes rounded. Young leaves have the upper surface closely beset with colourless jointed succulent hairs, which on mature leaves persist only along the margin, the upper surface becoming scabrid; lower surface glabrous. Scape glabrous, 12–18 cm. high, terminating in a compact head of 6–12 almost erect flowers. Bracts oval acute, sometimes toothed, margin finely beaded. Pedicels very short, 4–5 mm. Calyx tubular, 8 mm. long, cut to $\frac{1}{4}$ its length, the teeth acute. Corolla bright purplish pink, with yellow or crimson 'eye'. Tube 7 mm. long, limb 1.5 cm. across, the lobes obovate, sharply and regularly fimbriate. Seeds coffe-coloured, tuberculate.

A very distinct species. Its only near relations are *P. vaginata* and *P. eucyclia*. The creeping rootstock recalls the latter, but this species does not form mats, and is a much larger plant than *P. eucyclia*, with larger flowers and more of them, crimson instead of violet. The pedicels are much shorter than those of *P. vaginata*, the flower heads much larger.

Assam Frontier. Mishmi Hills. Delei valley, 28° 15' N., 96° 30' E. 9,000–10,000 ft. By streams in the forest, forming big colonies on steep shady faces. Flowers June.

K.W. 8346. *Primula septemloba*, Franch. var. *minor*, Ward (*Cortusoides*).

A smaller plant than *P. septemloba*, with fewer and smaller, very narrow tubular flowers. More hairy than *P. geraniifolia*, which it otherwise approaches very closely.

Assam Frontier. Mishmi Hills. Delei valley, 28° 15' N., 96° 30' E. On rocks in the forest, heavily shaded. 10,000–11,000 ft.

K.W. 8361. *Primula Agleniana*, Balf. f. et Forrest, var. *atrocrocea*, Ward.

A dwarfer plant than the type, not exceeding 6–10 in. Leaves not so laciniate. Flowers as large as, or larger than those of *P. Agleniana* in smaller heads, 2–3, rarely 5, of an intense gamboge yellow.

¹ Named in honour of my friend, Lady Norman.

Assam Frontier. Mishmi Hills. Delei valley, 28° 15' N., 96° 30' E. 12,000–13,000 ft. On alpine turf slopes, and along rocky alpine streams.

K.W. 8380. *Primula rubra*, Ward (*Sikkimensis*).

A glabrous perennial, 35–40 cm. high. Leaves distinctly petiolate, petiole as much as 14 cm. long; blade broadly ovate, or narrow ovate, about 9 cm. long, 7 cm. wide, the base cordate, margin irregularly lobed, the lobes ultimately finely toothed. Scape 35–40 cm. high ending in a head of 6–15 semi-erect flowers. Bracts acicular, about 1 cm. long, reddened. Pedicels short, 1–1.5 cm. Calyx narrow, 6 mm. long, cut $\frac{1}{3}$ of its length, teeth acuminate, scarcely angular. Corolla small, the tube yellow, 8 mm. long; the limb maroon red, 1.5–1.75 cm. in diameter, the lobes short, rounded.

A species allied to *P. firmipes* and *P. florindae*, and the first example of a red-flowered round-leaved species.

Assam Frontier, Mishmi Hills. Delei valley, 28° 15' N., 96° 30' E. 11,000–12,000 ft. In alpine meadows. Flowers June–July.

K.W. 8381. *Primula deleiensis*, Ward (*Sikkimensis*).

A dwarf plant, 20 cm. high. Leaves distinctly petiolate, petiole 4–5 cm. long; blade sub-rotund to oval, base shallow cordate 3 cm. long, 2 cm. wide, the margin irregularly dentate; the reticulate veining prominent below, shortly pilose. Scape slightly mealy below the inflorescence, which comprises 3–5 erect flowers on pedicels 2 cm. long. Bracts narrow linear acute, sometimes toothed, 7–8 mm. long. Corolla cream, 1.75 cm. long, limb spreading, about 2 cm. across, the lobes rounded with crimped or waved margin.

Perhaps a sub-species of *P. firmipes*, near *P. flexilipes*, from which it differs in its more dwarf habit, and fewer flowers on shorter pedicels, more upright, and cream coloured; also in the pilose veins of the under leaf surface.

Assam Frontier. Mishmi Hills. Delei valley, 28° 15' N., 96° 30' E. 11,000 ft. On alpine turf slopes, apparently very rare. Flowers June–July (earlier than *P. flexilipes*).

K.W. 8388. *Primula polonensis*, Ward (*Candelabra*).

A glabrous perennial up to 35 cm. high. Leaves as much as 25 cm. long, shortly petiolate, the blade tapering gradually to the sheathing stalk; narrow oval, about 20 cm. long, 6–8 cm. wide, the margin minutely dentate, the lower surface finely punctulate, giving a glistening effect. Scape ending in a head of more or less erect (drooping in bud) bright yellow flowers; a second whorl is produced later, and occasionally a third. Bracts acicular, up to 15 mm. long. Pedicels rather stiff, up to 3 cm. long. Calyx 1 cm. long, the teeth acute; sharply angular. Corolla 2 cm. long, about 2.2 cm. across, the lobes oval, 8 mm. long, 6 mm. wide. Seeds

small, black. A species closely allied to *P. Morsheadiana*, from which it differs remarkably in its root system. It is an altogether bigger plant than *P. Morsheadiana*, with larger and brighter flowers, and differently shaped leaves; a more robust grower.

Assam Frontier. Mishmi Hills. Delei valley, $28^{\circ}15'N.$, $96^{\circ}30'E.$ 9,000–10,000 ft. By streams in the forest, usually on outcrops of rock, associated with *P. Normaniana*, but much less common.

Professor W. Wright Smith, who has been over the material, kindly sends me the following notes on the five new species he recognizes. No. 8381, *P. deleiensis*, he regards as only a variety of *P. firmipes*, Balf. f. et Forrest. I have, however, retained its specific rank, having seen both plants in the wild state, and considering them sufficiently distinct for the reasons set forth.

P. rubra.

Species affinis *P. Waltoni*, Watt sed foliis cordatis inter alia recedit; haud remota est *P. firmipes*, Balf. f. et Forrest cujus flores flavi nec rubidi.

P. Clutterbuckii.

Proxima *P. yunnanensi* Franch. a qua calyce valde elongato, lobis linearibus flaccidis recedit.

P. Normaniana.

Species affinis *P. vaginatae*, Watt atque *P. eucycliae*, W. W. Smith et Forrest a quibus habitu robustiore, scapo longiore, floribus multis congestis (pedicellis brevissimis) corollae lobis valde fimbriatis differt.

P. mishmiensis.

Planta affinis *P. callianthae*, Franch. et *P. bryophilae*, Balf. f. et Farrer a quibus floribus flavis cognoscitur.

P. polonensis.

Species affinis *P. Morsheadianae*, Ward a qua habitu robustiore majoribus, corollae annulo valde distincto recedit.

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Contributions to the Study of *Humaria granulata*, Quel.

BY

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AND

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With Plates XIII and XIV and ten Figures in the Text.

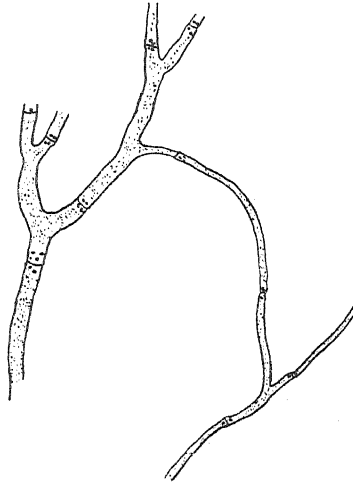
HUMARIA GRANULATA Quel. is a member of the Pezizaceae with orange-coloured ascocarps, one to five millimetres in diameter, occurring not uncommonly on cow dung at all seasons of the year. The development of the fructification was described by one of us jointly with Professor V. H. Blackman in 1906 (7); an account of the ascus was published in 1909 (9). The material for both these investigations grew on the natural substratum. It appeared to us that, if the fungus could be brought into culture, further details of the development of the ascocarp might perhaps be made available.

In June and October, 1926, wild material was accordingly collected and attempts were made to germinate the spores. Some were placed in centinormal solution of sodium carbonate at 30° C. for 18 to 24 hours. For others agar media were used, especially 3 per cent. agar made up with cold water extract of cow dung to which a little sodium carbonate solution had been added, and small pieces of grass as a source of carbohydrate. This medium was largely used throughout our investigations, and will be designated as *cow dung agar*. In some cases the spores were exposed to temperatures of from 70° to 150° C. for varying periods and incubated at 30° C. for several days; in others they were kept at 48.5° C. for three hours, and left in a sunny window at 20° C. In no case was there any sign of germination.

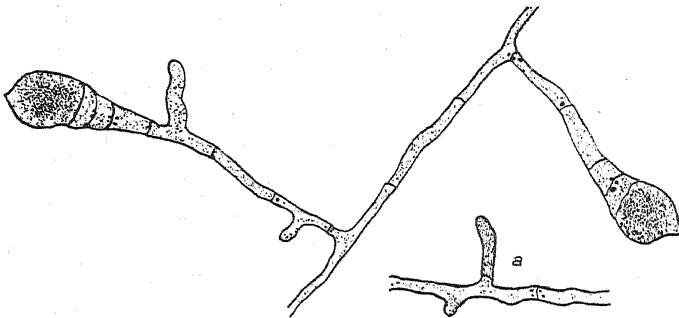
Plates were therefore inoculated with single ascocarps or scraps of ascocarp wall, and, after a number of transfers, clean mycelia were obtained and gave rise to fruits.

Material was fixed for the most part in Flemming's strong fluid

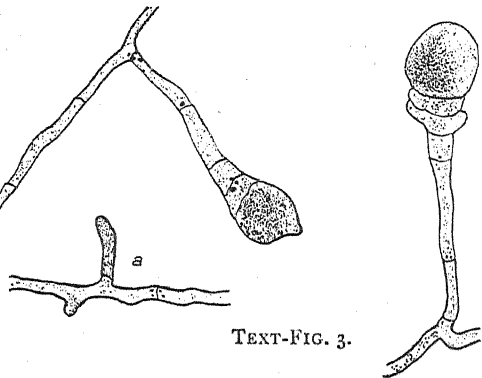
diluted with an equal quantity of water, and stained with iron alum haematoxylin followed by erythrosin in clove oil, or in special cases with Breinl's combination. We found the results in the case of haematoxylin



TEXT-FIG. 1. Anastomosis of hyphae in a single spore culture. $\times 300$.



TEXT-FIG. 2.



TEXT-FIG. 3.

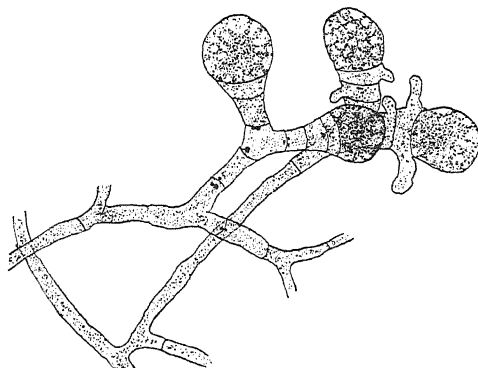
TEXT-FIG. 2. Young archicarps on mycelium (L_4) from a + spore; a very early stage of development is shown at *a*. $\times 300$.

TEXT-FIG. 3. Young archicarp on mycelium (N_1) from a - spore. $\times 300$.

greatly improved when the material, after 24 hours' staining was rapidly washed out in about 8 per cent. iron alum. This was made up fresh on each occasion by adding one or more crystals of iron alum and two or three drops of glacial acetic acid to a Petri dish of distilled water.

Mycelium. The mycelium of *H. granulata* consists of rather long, multinucleate cells separated by walls on which groups of deeply staining granules are often seen (Text-fig. 1); similar granules may be found in the body of the cell, and are especially noticeable in the oogonium,

both lying free and in contact with the basal wall. Dichotomous branching is frequent, and smaller, lateral branches are also given off. Anastomoses between neighbouring hyphae readily occur. Conidia are not produced. Occasionally tangles of sterile hyphae are found; these have not been observed to fulfil any function and seem to be of the nature of abortive fruits; in most cases they do not possess an archicarp.



TEXT-FIG. 4. Branched archicarps on mycelium (L_4) from a + spore. $\times 300$.

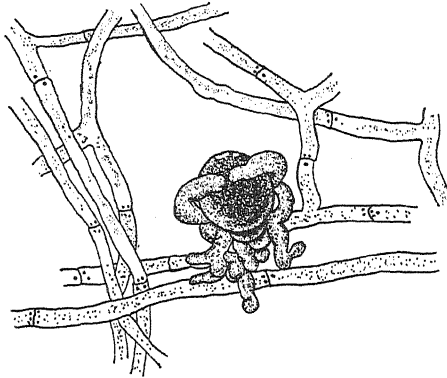
Archicarp. The young archicarp is first recognized as a slightly swollen hypha, the distal end of which contains reticulate cytoplasm and numerous nuclei (Pl. XIII, Fig. 1). Such hyphae may be borne singly (Text-figs. 2, 3) at any point on the active mycelium, or may arise in pairs from a common base (Text-figs. 4, 9). The latter arrangement recalls the dichotomously branching primordia of *Ascodesmis* (3) and *Pyronema* (1, 12). There is no sign of an antheridium at any stage of development. The young archicarp undergoes septation, the free end becoming enlarged to form a globular oogonium. The growth of the transverse walls appears to be annular; a central pore remains for some time (Pl. XIII, Fig. 2), and the granules on the wall are probably associated with its closure.

In every culture a number of unsuccessful archicarps may be noticed (Text-fig. 9), becoming emptied of contents and failing to grow beyond the uncovered stage. Contents disappear first from the stalk (Pl. XIII, Fig. 4), the sheath fails to develop, and later the oogonium is found to be empty. This appearance suggests starvation, which may, to some extent, be due to overcrowding. Such archicarps are shown on the natural substratum by Blackman and Fraser (7) in their Figs. 2 *a*, 2 *b*, and 3.

The Sheath. As growth proceeds branching takes place in the cells of the stalk, and especially in those nearest the oogonium (Pl. XIII, Fig. 3); the branches at first form a frill beneath the oogonium (Pl. XIII, Fig. 5), soon they extend around and above it (Pl. XIII, Fig. 6), constituting the first layers of the sheath. One or two branches are apt to outgrow the others

and fold across the oogonium (Text-fig. 5), they can sometimes be recognized at a later period (Pl. XIII, Figs. 9, 10). In the early stages at any rate the sheath is wholly derived from the cells of the stalk.

The Oogonium. The young oogonium is crowded with nuclei (Pl. XIII, Fig. 5), later, as it grows, the nuclei are not sufficiently numerous to fill the increasing area, and are withdrawn from the central region, lining the walls in an irregular series two or three deep (Pl. XIII, Fig. 6). At this time



TEXT-FIG. 5. Archicarp in mixed culture showing sheath in process of development. $\times 300$.

the main part of the sheath is about half way up the oogonium; by the time it has closed in the whole area is again crowded with nuclei. Nuclear division is karyokinetic and occurs simultaneously in all nuclei of an oogonium (Pl. XIII, Fig. 7). It is not often seen and may be inferred to take place with some rapidity. Our best figures were obtained in material fixed in the month of July at 1.15 p.m. and 10 p.m., Greenwich mean time.

The tip of the oogonium shown in Pl. XIII, Fig. 1, contains 42 nuclei; in oogonia at the stages of Pl. XIII, Figs 2, 3, and 5, taking all sections into account, from 164 to 364 have been recorded, while full-grown oogonia, just before the ascogenous hyphae begin to form, reveal from 593 to 1,408 nuclei. These numbers suggest that two divisions take place before the oogonium is mature. Associated with the difference in nuclear content of mature oogonia is a wide divergence in size; the largest oogonium measured had an average diameter of 59μ , while the average diameter of the smallest single oogonium was no more than 38μ ; in the case of a pair of oogonia on a branched stalk the average diameters were 33μ and 34μ . We are inclined to attribute these variations to some extent to the occurrence of branched archicarps, and to infer that, when branching has taken place, the available nuclei are distributed between the two oogonia. The development to maturity of both oogonia is, however, rare, perhaps because, when nuclear distribution is unequal, only one receives enough for efficient growth.

In a fully formed oogonium the resting nuclei are remarkably uniform

both in size and appearance; the stainable material is collected in a globular mass surrounded by a clear area with a definite boundary. The two last-named characters distinguish the nuclei from the granules which are often present, the latter, moreover, stain deeply throughout, and are, for the most part, considerably larger than the stainable chromatin mass.

During the next stage of development, when the ascogenous hyphae are preparing to bud out, oogonia contain both nuclei of about the same size as hitherto and nuclei noticeably larger. Cases may be observed in which two of the smaller nuclei are pressed close together, in other cases a single nuclear area surrounds two chromatin masses (Pl. XIII, Fig. 8 *a*; *b*), while in others again two chromatin masses are in contact in a common area (Pl. XIII, Fig. 8 *c*). We are unable to interpret these appearances otherwise than as stages in nuclear fusion. They are not pathological, as suggested by Ramlow (11) for *Ascophanus carneus*, since they occur in healthy oogonia in full activity; they are not phases of nuclear division, since, as already stated, this takes place in all nuclei of the oogonium at the same time and gives quite a different appearance; they are never found in respect of the larger, but only of the smaller nuclei. Fusion appears to be associated with the entrance of nuclei into the ascogenous hyphae; there is no massing of the nuclei in the oogonium such as occurs in *Pyronema confluens* (1) and *Pyronema domesticum* (19), when the male nuclei are passing into the female organ. Our observations confirm those of Blackman and Fraser (7).

It may be noted that the stages of nuclear division and fusion are very easily missed; not only is rapid fixation at a time of full activity essential, together with most careful staining, but critical illumination is required. With a 'fullolight' electric lamp, for example, which is excellent for general purposes, it was impossible to be sure of the inter-relationship of the nuclei; when a gas flame impinging on a thorium disc was used as a source of illumination the common nuclear area and the contact of chromatin masses could with certainty be seen.

A little later, as the development of the ascogenous hyphae proceeds, the disparity in the size of the nuclei in the oogonium ceases, an occasional small nucleus only being observed; this is already the case in the oogonia shown in Pl. XIII, Figs. 9 and 10. Counts were made of the nuclei in a series of oogonia and their ascogenous hyphae at this stage. The numbers ranged from 273 to 685, the lowest being found in the pair of oogonia on a branched stalk to which reference has already been made and which showed 273 and 294 respectively. The nuclei are easier to count now than when, rather earlier, they are crowded together in the oogonium, since they are more spread out, some having entered the ascogenous hyphae, and there is less danger of individuals being overlooked. Such counts confirm the evidence of those taken earlier in development that the oogonia vary considerably in content as well as in size, but, even when allowance is made for this and for

a reasonable margin of error, they indicate a diminution in the number of nuclei which, since no signs of disintegration are seen, can only be due to fusion.

TABLE I.

	No. of Oogonia.	Total no. of Nuclei.	Largest no. of Nuclei.	Smallest no. of Nuclei.	Average in Whole Numbers.
Oogonia containing only small nuclei	14	12,385	1,408	593	884
Oogonia forming ascogenous hyphae	14	6,159	685	273	440

In oogonia containing both large and small nuclei, intermediate numbers, ranging from 943 to 449 were found.¹

The Ascogenous Hyphae. The formation of ascogenous hyphae begins when the sheath is only one or two layers thick. Sometimes the hyphae extend well beyond the limits of the sheath (Pl. XIII, Fig. 9), but for the most part they ramify among its filaments (Pl. XIII, Fig. 10), and they are soon completely enclosed within its limits (Pl. XIII, Fig. 11). They branch freely, often by an abrupt dichotomy; their ends are often curled over, recalling the familiar crozier formation of the hymenium, their walls are at first more delicate than those of the surrounding cells, so that, as they grow among them, they are moulded to their form. They are readily traced by the fact that their contents are denser than those of the sheath cells, and their nuclei larger and better marked.

The nuclei enter the ascogenous hyphae in single file (Pl. XIII, Fig. 9), sometimes two or three nuclei lie close together, but there is no evidence at this stage of any association between them.

When part of the contents of an oogonium has passed into an ascogenous hypha a vacuole remains (Pl. XIII, Figs. 9, 10); the oogonium is thus gradually drained of contents, only a little disintegrating cytoplasm and a few nuclei being eventually left behind.

During the growth of the ascogenous hyphae karyokinesis again occurs, taking place in all ascogenous hyphae simultaneously (Pl. XIV, Fig. 12), as well as in all active nuclei remaining in the oogonium (Pl. XIV, Fig. 13). Throughout the ascocarp the same stage of division is observed in every nucleus; the chromosomes, though small, are clear, and are found to be four in number. In the fructification from which Figs. 12 and 13 were taken, and also in that which gave Figs. 14 and 15, the development of the ascogenous hyphae is well advanced, but there is some evidence that an earlier division also occurs, at a stage between that of Figs. 10 and 11; the close proximity of

¹ The details of the counts for the twenty-eight oogonia were as follows: Oogonia containing small nuclei only, 710, 978, 828, 593, 663, 637, 832, 1,408, 918, 1,119, 1,195, 1,000, 817, 687. Oogonia with ascogenous hyphae, 348, 602, 273, 294, 326, 685, 512, 449, 351, 597, 507, 410, 392, 413.

many of the nuclei in the ascogenous hyphae of Fig. 12 and neighbouring sections also suggests that they may be sister nuclei of an earlier mitosis.

The Ascus. The development of the ascus takes place along the familiar lines. The tip of the ascogenous hypha bends over, nuclear division occurs, the ascus is derived from the penultimate, binucleate cell, and in it two nuclei fuse. We observed no correlation at this or later stages between the occurrence of karyokinesis in neighbouring cells. No doubt by this time the ascogenous hyphae have lost their connexion with the oogonium and with one another. In the first division of the definitive nucleus eight gemini are seen (Pl. XIV, Figs. 16, 17), and eight chromosomes travel to each pole of the spindle (Pl. XIV, Fig. 18). Having in mind Tandy's investigation of *Pyronema domesticum* (19), in which the definitive nucleus is diploid in some asci and tetraploid in others, we instituted a search for variations in chromosome number, but in all cases that came under our observation the number was the same.

In the second division the number of chromatin masses is no longer eight, but four (Pl. XIV, Fig. 19); four chromatin masses pass to each pole, four appear once more in the third prophase, and four are distributed (Pl. XIV, Fig. 20) to the nucleus of each ascospore.

These observations confirm the statements of Fraser and Brooks (9). As the development of the ascus is fully illustrated in that paper, we have figured only the most critical stages, those in which eight gemini or four or sixteen chromatin masses appear. In these small nuclei eight chromosomes spread along a spindle may be interpreted either as the metaphase in a nucleus with eight chromosomes, or as the early anaphase in a nucleus with four. Such stages, therefore, have no critical significance.

The Chromosome Number. Since the nucleus of the ascospore has four chromosomes, it is evident that four is the haploid number, and the definitive nucleus of the ascus, having eight gemini, must be regarded as tetraploid. This indicates the occurrence of two fusions between the germination of the ascospore and the incidence of meiosis. One nuclear fusion has been observed in the oogonium, the second in the developing ascus. Under these circumstances the presence in the ascogenous hyphae of nuclei with four chromatin masses demands explanation; we are inclined to attribute it to the premature association of the allelomorphs, an association which is continued through the fusion in the ascus and the meiotic pro-phases; it ends on the spindle of the heterotype division, when eight single chromosomes are observed travelling to each pole, and again occurs in the prophase of the second division in the ascus, where four chromatin masses appear. This would imply that two allelomorphs tend to unite whenever they are present in the same resting nucleus, but that four do not enter into such association. Unfortunately the nuclei of *H. granulata* are too small for the chiasmotypic stages to repay investigation. Premature

association of the allelomorphs was recorded by Harper (5) in the sexual nuclei of *Pyllactinia corylea* in 1905, and in the ascus of various fungi by Maire (6), Guilliermond (4) and Fraser and Welsford (8).

If the nuclear divisions alone were considered the chromosome numbers of *H. granulata* might perhaps be explained by the assumption that the nuclei of the ascogenous hyphae, with four chromatin masses each, are still haploid, and that the eight masses passing to each pole in the heterotype anaphase are four chromosomes in which the longitudinal fission has early been completed. Against this is the fact that four chromatin masses again appear in the succeeding homotype prophase, demanding the difficult assumption that the longitudinally split halves have united again. Nor will this hypothesis explain the appearance of eight chromatin bodies in the first prophase in the ascus, where the alternative is offered of the failure of the gemini to be formed or of a longitudinal split which coincides with their formation. We find the early association of the allelomorphs a more probable hypothesis, and we note that it accords with the observed occurrence of nuclear fusion in the oogonium, as the suggestion of haploid nuclei in the ascogenous hyphae does not.

But, whatever their true explanation, the facts remain that, in the material under investigation, four chromatin bodies are found in the ascospore nuclei, four in the ascogenous hyphae, and eight in the definitive nucleus of the ascus after meiosis has begun, when a diploid nucleus would show four, the haploid number.

The Germination of the Ascospores. During the progress of these investigations renewed attempts were made from time to time to bring about the germination of the spores. As soon as fruits were available which had been grown in culture, and were therefore not contaminated with other organisms, as those on the natural substratum must be, spores were removed, heated to various temperatures and placed on various media, but without result. Later, in consequence of our experience with *Lachnea cretea* (17), spores which had been shot from the ascus on to the surrounding medium were employed. A drop of centinormal aqueous solution of sodium carbonate was placed on the agar, the spores were drawn up in a fine capillary tube and transferred to a new plate; a drop of centinormal sodium carbonate was added, and the spores spread out with a wire. Temperatures found useful in the case of other fungi were employed, but without result. The culture used in these experiments had been inoculated on January 18, 1927; it had fruited on February 9, 1927; subsequent experiments showed that the spores are shot about nine days after the appearance of the fruits; the spores were taken in May.

On July 4, 1927, further groups of spores from the same culture were spread on cow dung agar by the method described above. Plates were incubated at 38° C. for 18 hours and left at 23° C., but no germinations

ensued; others were raised to 48.5° C. for 2 hours and placed at 23° C., they showed germination in 48 hours. Ascospores incubated at 60° for 15 minutes, 70° for 15 minutes, or 80° for 10 minutes, followed by 30° showed no germination.

On July 11 ascospores treated in the same way as those which had germinated after 48 hours were found to germinate after 24 hours, whether in light or darkness.

Germination thus occurred after a heat stimulus in spores 21 and 22 weeks (5 months) old. Spores 2, 3, and 4 months old were tested without result.

On September 14, 1927, when the spores shed in February were 7 months old, some of them were used to inoculate plates of cow dung agar, which were kept in the window at 16° to 18° C.; these germinated after 24 hours, without the application of heat.

On February 22, 1928, a series of inoculations was made from spores a year old. Germination was slow, and did not take place in all plates, indicating that, by this time, some vitality had been lost. Spores on plates at 18° to 20° C. germinated 10 days after inoculation.

In July, 1927, as soon as it was realized that a period of maturation must elapse before the spores could germinate, fruiting cultures were set aside and became available in February, 1928, as a source of active spores. From these, single spore cultures were obtained. Spores were allowed to germinate in the way already described and single, germinating spores were transferred to fresh plates of cow dung agar.

The mycelium from single spores was healthy and gave rise to numerous archicarps (Text-figs. 2, 3, 4), the oogonium was cut off and enlarged, the stalk cells began to branch, but the branches never covered the oogonium, ascogenous hyphae were not put out, and fruits failed to develop.

Cross Inoculation. The experiment was then tried of inoculating plates with a single spore on either side. Where two mycelia met across the middle of the plate a line of healthy fruits developed. A series of cross inoculations, examples of which are shown in the following table, made evident that the mycelia are of two kinds, which were arbitrarily termed + and —, no structural difference being found between them. The + mycelium is distinguished only by the fact that it forms fruits when it meets the mycelium from a — spore, and the — only by the fact that it forms fruits with a +. The + and — characters proved to be perfectly constant, no intermediate forms were found, nor did the character disappear or alter with the passage of time. It is present in all mycelia from a given spore, and may reasonably be regarded as hereditary.

With a view to ascertaining whether the distinction between + and — mycelia was affected by nutrition, a number of attempts were made to grow

the fungus on synthetic agars. Claussen's medium (10) and Barnes's medium (16) were tried as well as the media known as 'M' (0.1 per cent. K_3PO_4 , 0.1 per cent. NH_4NO_3 , 0.1 per cent. $MgSO_4$) with and without 0.2 per cent. glucose, and a modification of the last named consisting of 0.1 per cent. K_3PO_4 , 0.05 per cent. NH_4NO_3 , 0.05 per cent. KNO_3 , 0.1 per cent. $MgSO_4$. Both single spore mycelia and cross inoculations were employed, but, though a fair growth of mycelium was obtained on the 'M' media, and apparently healthy archicarps sometimes appeared at the point of contact of + and - strains, fruits never matured.

TABLE II.

	L_4	R_1	S_1	S_2	S_5	R_2	S_3	P_5	M_2
L_4	O	O	O	O	O	F	F	F	F
R_1	O		O	O	O	F	F	F	F
S_1	O	O		O	O	F	F	F	F
S_2	O	O	O		O	F	F	F	F
S_5	O	O	O	O	O	F	F	F	F
R_2	F	F	F	F	F		O	O	O
S_3	F	F	F	F	F	O		O	O
P_5	F	F	F	F	F	O	O		O
M_2	F	F	F	F	F	O	O	O	O

F indicates the formation of fruits and O their absence; L_4 and mycelia with the same reaction were described as +.

On media of uncertain constitution, such as cow dung agar, no attempt could be made to study the effect of simple ingredients, but, since it seemed possible that the enzyme or vitamin content of + and - mycelia might differ, cow dung agar was made up with Bemax, a preparation rich in vitamin B. We are glad to take this opportunity of expressing our cordial thanks to Dr. J. M. Rowlands for the useful information that, when Bemax is subjected to a dry heat of $200^\circ C.$, less than 25 per cent. of the vitamin is destroyed. Accordingly Petri dishes containing Bemax were raised to 200° in a dry oven and cooled under sterile conditions. Sterilized cow dung agar, just warm enough to be still fluid was then poured in. Both + and - mycelia grew well, but neither alone gave rise to archicarps.

Recalling that Stevens (23) had successfully induced the formation of fruits by subjecting material of *Glomerella cingulata* to the action of ultra violet light, we employed radiations from a mercury vapour lamp both on monosporous cultures, and on plates inoculated with two + or two - mycelia. Petri dishes, with the lids removed, were left for 10 seconds at a

distance of 30 cm. from the source of light, for 1 minute at a distance of 100 cm., and for 5 minutes at a distance of 200 cm., but in every case without effect. In several instances successful sub-cultures were made after treatment, proving that the mycelium had not been destroyed. Ergosterol has been shown to be the substance from which vitamin D is formed by irradiation (22) and, as its presence in the culture used is a matter of doubt, further investigation is proceeding along these lines, and also in the direction of the employment of shorter exposures.

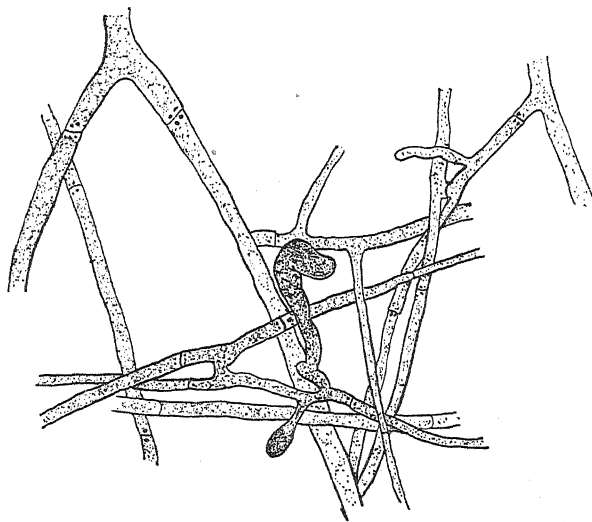


TEXT-FIG. 6. Archicarp from a plate infected with two — spores; the maximum development found in single strain cultures has been reached; a few branches have been put out, a number of mycelial fusions have occurred. $\times 300$.

The Union of + and — Strains. Meanwhile a detailed study was undertaken both of the monosporous mycelia and of the line of junction between mycelia from different spores. For this purpose sections are not satisfactory; pieces of agar, about 2 inches long by 1 inch wide were fixed in the usual way, washed in running water, taken up through spirit to Calberla's fluid, and transferred to equal parts of pure glycerine and saturated aqueous solution of erythrosin. Here they remained for 48 hours before washing in 50 per cent. and 75 per cent. aqueous glycerine. By this treatment the fungus is stained a brilliant red and the agar pale pink, so that all ramifications of the mycelium can readily be traced. A thin surface section of the whole piece of agar was taken with the blade of a safety razor, gently warmed, mounted in glycerine jelly and covered with a large cover slip. The warming softens the agar and ensures its incorporation with the jelly. An objection to this method is that the mycelium, as it grows, is apt to bend downwards through the agar and thus be lost when the top slice is taken. A thick section is useless, as its surface only can be focused with a one-sixth objective. To obviate these difficulties the middle region, about 1 inch wide, was cut out across a plate of agar and replaced by a very thin film of the medium raised on an ordinary microscope slide. As a rule this

was done after the first row of fruits had appeared from cross inoculation, and thus a second crop was obtained. Under these conditions growth took place nearly in one plane.

In single strain cultures, that is, in cultures from + material or — material only, whether monosporous or no, frequent anastomoses occur of vegetative hyphae (Text-fig. 1), uncovered archicarps are formed which



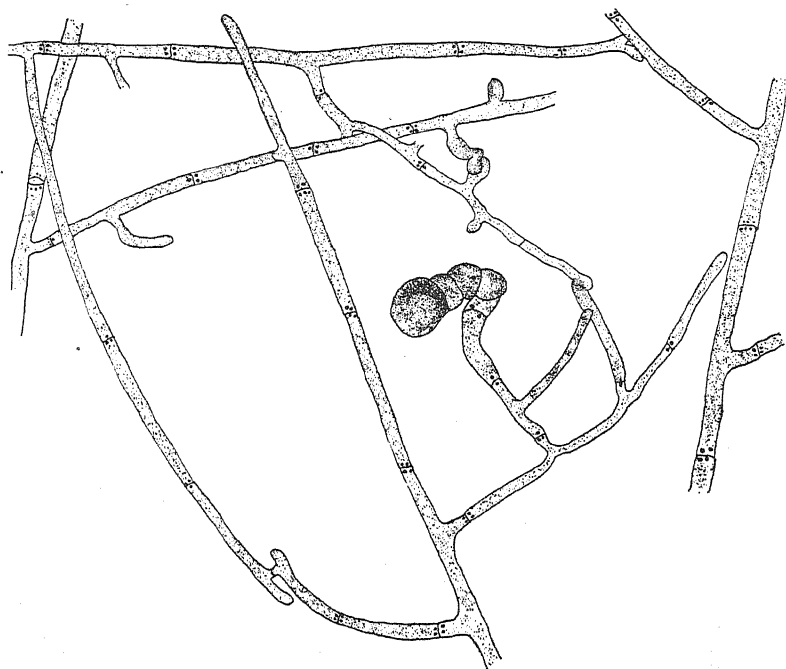
TEXT-FIG. 7. Young archicarp at the junction of + and — mycelia; anastomoses have taken place near its point of origin. $\times 300$.

appear entirely healthy and normal (Text-figs. 2, 3, 4), they put out the first branches of the sheath (Text-fig. 6). Later, however, development fails and, after several mycelial fusions have taken place (Text-fig. 6), perhaps in search of food, the archicarp becomes empty of content and at last degenerates. Occasionally, in cultures of a single strain, wefts of hyphae may be found, such as have been described above for mixed cultures. Superficially they resemble young ascocarps, but they lack normal contents. In one case only, among the many single strain cultures we have grown, were these structures large enough to be seen with the naked eye, giving the appearance of pale fruits; microscopic examination, however, showed them to be without ascogenous hyphae or asci.

At the point of junction of hyphae from a + and a — spore, numerous mycelial fusions occur, and, in the neighbourhood of these, archicarps develop and not only reach the uncovered stage (Text-figs. 7, 8), but give rise to ascogenous hyphae and ultimately to ascospores. Text-fig. 9 is drawn from material of the same age as that represented in Text-fig. 10, but the former comes from the junction of + and — strains, and the latter

from a plate inoculated with two — mycelia. Even in the mixed culture a few unsuccessful archicarps are to be seen.

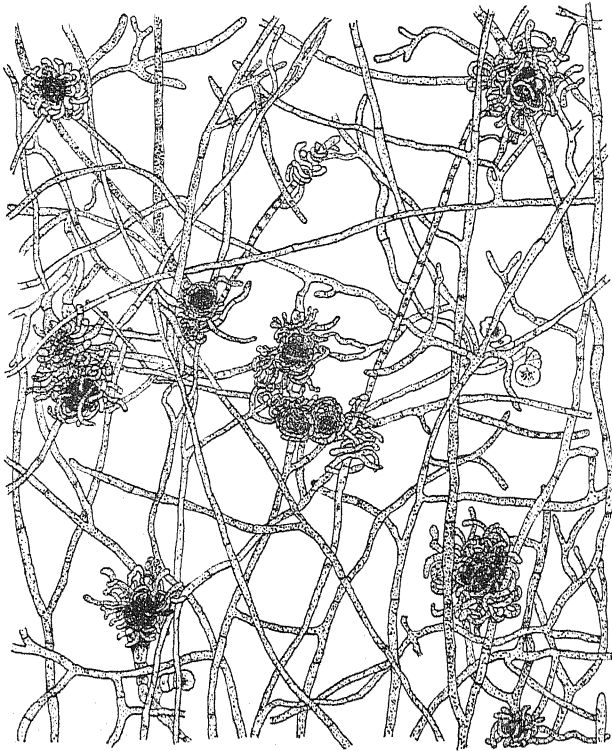
Distribution of + and — Spores. In order to ascertain whether the same ascocarp produces + and — spores, single ascocarps, when fully grown, were removed each into a small, sterile tube, and left the necessary months for



TEXT-FIG. 8. Archicarp developing in the neighbourhood of anastomoses between + and — mycelia. $\times 300$.

the spores to mature. In April, 1929, spores from single ascocarps were placed on cow dung agar in a drop of centinormal sodium carbonate solution, well spread out, incubated at 48.5°C . for two hours and left at 18°C . Abundant germinations occurred, and single spores, single asci, groups of spores and groups of asci were separated on to fresh plates of cow dung agar. Cultures from groups of spores or groups of asci from the same ascocarp fruited readily; single spore cultures, when grown against each other, gave a line of fruits in the usual proportion of cases. Owing to the long maturation period of the spores, it was unfortunately not possible to separate spores in series from a single ascus. After seven months the group shot from an ascus has lost its coherence, or, if the spores are still retained in the ascus, they are attached to its wall and cannot be broken apart. In four cases a single ascus was isolated containing germinating spores, in each of these the culture gave rise to fruits.

From these observations it is evident that the ascocarp of *H. granulata* produces both + and - spores, and that an ascus, in some cases at any rate, contains spores of both kinds. When it is remembered that each fruit arises from a single archicarp, and that the only connexion between the mycelium and the ascogenous hyphae is through the oogonium, it

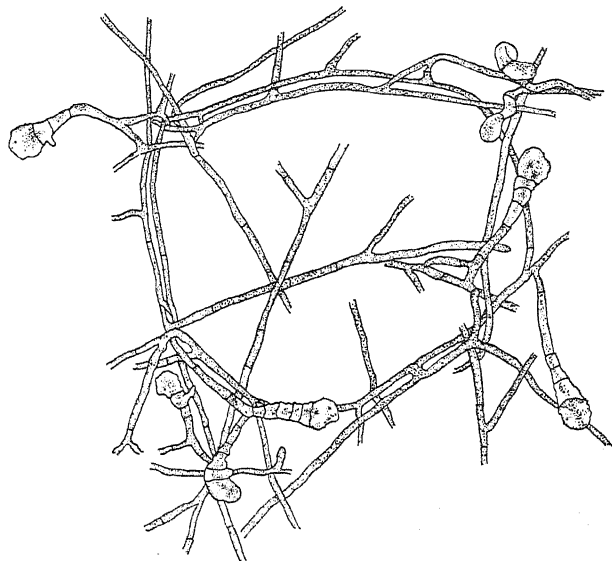


TEXT-FIG. 9. Group of immature ascocarps from a mixed culture fixed seven days after inoculation. In several cases the archicarps are branched; at the bottom left-hand corner one member only of such a pair has developed; towards the middle of the right-hand side both have failed to do so. In the upper middle region is one of the hyphal tangles referred to on pp. 129 and 138; another occurs rather lower down. $\times 110$.

becomes clear that material from both + and - thalli must have passed into the archicarp, and it is inevitable to infer that the transmission of the + and - characters is accomplished by means of the nuclei. Further, since the ascus, at one stage of development, contains a single nucleus, and since the same ascus may produce both + and - spores, it cannot be doubted that fusion between + and - nuclei takes place. A similar condition has been described by Wilcox for *Neurospora sitophila* (23), a form in which heterothallism was first observed by Shear and Dodge (18), but there all the spores in an ascus have been germinated, and individual asci have been shown to contain four + and four - spores. Where all asci

contain + and - spores in equal number selective nuclear fusion may be inferred to have taken place.

In *H. granulata* there is no evidence in regard to the selective nature of the fusion either in the oogonium or in the ascus. Supposing nuclear union to take place at random in both cases, the tetraploid nucleus of the ascus will contain + and - characters in accordance with the equation



TEXT-FIG. 10. Material of the same age as that in Text-fig. 9, but from a plate inoculated with two - mycelia; a number of dying archicarps are seen. $\times 110$.

$(a+b)^4 = a^4 + 4a^3b + 6a^2b^2 + 4ab^3 + b^4$. That is to say, out of every sixteen asci, two may be expected to contain + spores or - spores only, eight to contain six + or six - spores and two of the other strain, and six to contain + and - spores in equal number. The proportion of asci containing spores of both strains to those containing one type of spore will be as seven to one. Were there but one fusion, without selection, the expectation would be in accordance with the formula $a^2 + 2ab + b^2$, only half the asci containing + and - spores. In heterothallic forms in which fusion occurs without selection, there may perhaps be some advantage in the retention of the apogamous fusion in the oogonium.

Heterothallism has been recorded in a number of other ascomycetous fungi, but in none has the behaviour of the spores been so thoroughly studied as in *Neurospora* (20, 24), and in none is information available on the morphology of the mycelial union. The position in this and other groups of fungi has recently been reviewed by one of us (21), and sufficient new material is not available to justify reconsideration here.

The Significance of Heterothallism. Since Blakeslee's discovery in

1904 (2) of the condition in moulds which he defined as heterothallic, the term has come to be used by many authors to indicate dioecism in the gametophyte. On the etymology of the word this use is justified, for the dioecious gametophyte has indeed thalli of different kinds. In *H. granulata*, however, there is no sexual difference, all thalli bearing oogonia, and being, therefore, either monoecious or of the female sex. It is true that antheridia are not produced, but, since they are found associated with similar oogonia both in *Pyronema* (1, 10, 19) and in *Ascobolus* (12), the nature of that organ in *Humaria* is not in doubt.

But, though all thalli be monoecious in *Humaria*, all thalli are not the same, and the female organ is incapable of giving rise to the ascogenous hyphae which constitute the sporophyte, until fusion of two appropriate thalli occurs. Here is a condition which may properly be described as heterothallic, but which is not sexual heterothallism. Since the oogonium, in the absence of mycelial fusion, becomes empty, shrivels and dies, the condition may tentatively be described as nutritive heterothallism, but we have not yet been able experimentally to justify this term.

Part of the mycelium in a mixed growth of *H. granulata* is derived from single spores, part is due to the combination of two thalli; it is convenient to be able briefly to express these conditions. The mycelia of a single strain will therefore be described as *monothallic*, and those derived from the fusion of + and - filaments as *dithallic*. These terms correspond in significance with the terms primary and secondary as applied to the mycelium of heterothallic Basidiomycetes, but the use of secondary in the sense in question is not admissible among Ascomycetes, since the word has long been employed to denote the branches which grow down from a developing ascocarp to obtain food.

The functional archicarps and oogonia, then, of *H. granulata* are dithallic, and so are the ascogenous hyphae and the asci themselves. The separation of + and - characters takes place in the nuclear divisions in the ascus; the resultant spore nuclei are either + or -, and the ascospores are therefore monothallic. The ordinary mycelium also is monothallic till the anastomosis of + and - hyphae takes place.

In addition to the oogonium and its sporophytic products, the archicarp gives rise by lateral branching to the filaments which constitute the sheath. These, being derived from the stalk of the archicarp, and not from the oogonium, are part of the gametophyte, but, like the sporophytic hyphae, they are dithallic. The whole ascocarp, in fact, is derived from the union of + and - mycelia, and is formed of dithallic cells. In this it resembles the sporophore of the *Coprini*, which is built of dithallic (or secondary) mycelium, but which differs from the ascocarp of *Humaria* in lacking any indication of gametangia or of a sporophyte derived from them. In the absence of sexual organs there is no evidence that the heterothallism

of the Hymenomycetes is of a sexual rather than a nutritive type. The fact that, in some species, mycelia have been observed of four strains (13, 14), and the fact that, in others, spontaneous development of the dithallic mycelium may occur (15, 25), suggest, indeed, that the phenomena are not those of sex as usually understood.

In the Zygomycetes, on the other hand, gametangia are formed, as in *H. granulata* both on the + and on the — mycelium. But here the gametangia unite and the dithallic product is synonymous with the sporophyte.

Humaria shows well-developed nutritive heterothallism while retaining recognizable evidence of sex. The investigation of the sexual apparatus in other heterothallic forms is greatly to be desired.

SUMMARY.

1. In *H. granulata* the ascospores germinate with the help of a heat stimulus five months after they are shed. They have not been induced to germinate earlier. After seven months they germinate without stimulation; they retain their vitality for over a year.

2. The spores and the mycelia to which they give rise are of two kinds, distinguished as + and —. There is no morphological or growth difference between them. Both + and — mycelia show fusions between their branches and produce archicarps which cut off oogonia. Further development of the oogonia does not take place in monosporous culture.

3. Where + and — mycelia meet, anastomoses are abundant. In the neighbourhood of such fusions the development of the archicarp is continued; the stalk gives rise to branches forming the sheath, the oogonium puts out ascogenous hyphae. Both the sporophytic ascogenous hyphae and the filaments of the sheath, which form part of the gametophyte, are *dithallic*, being the outcome of the union of two mycelia. The product of a single spore is described as *monothallic*.

4. There is no antheridium; the development of the oogonium is apogamous; nuclei fuse in pairs before the ascogenous hyphae are put out. After the fusion stage the nuclei in the oogonium and its branches are larger than before, and only about half as numerous.

5. Nuclear division in the oogonium is karyokinetic and occurs in all nuclei at the same time. Two mitoses occur before the ascogenous hyphae are formed, and at least one during their development, while they are still attached to the oogonium.

6. The definitive nucleus of the ascus shows eight gemini, and eight chromosomes pass to each pole in the first meiotic division. In the second division only four chromatin masses are seen, and in the third division four are distributed to each ascospore nucleus. Four is the haploid number. The presence of four chromatin masses in the second division in the ascus

indicates an association of allelomorphs; the same thing is found in the ascogenous hyphae, where four chromatin masses also appear.

7. The same ascus, in the cases examined, contained both + and — spores. It was not possible to ascertain the proportion of each or their arrangement in the ascus.

8. *H. granulata* shows well-marked nutritive heterothallism while retaining recognizable evidences of sex.

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EXPLANATION OF PLATES XIII AND XIV.

Illustrating Professor Gwynne-Vaughan and Mrs. Williamson's paper on *Humaria granulata*, Quel.

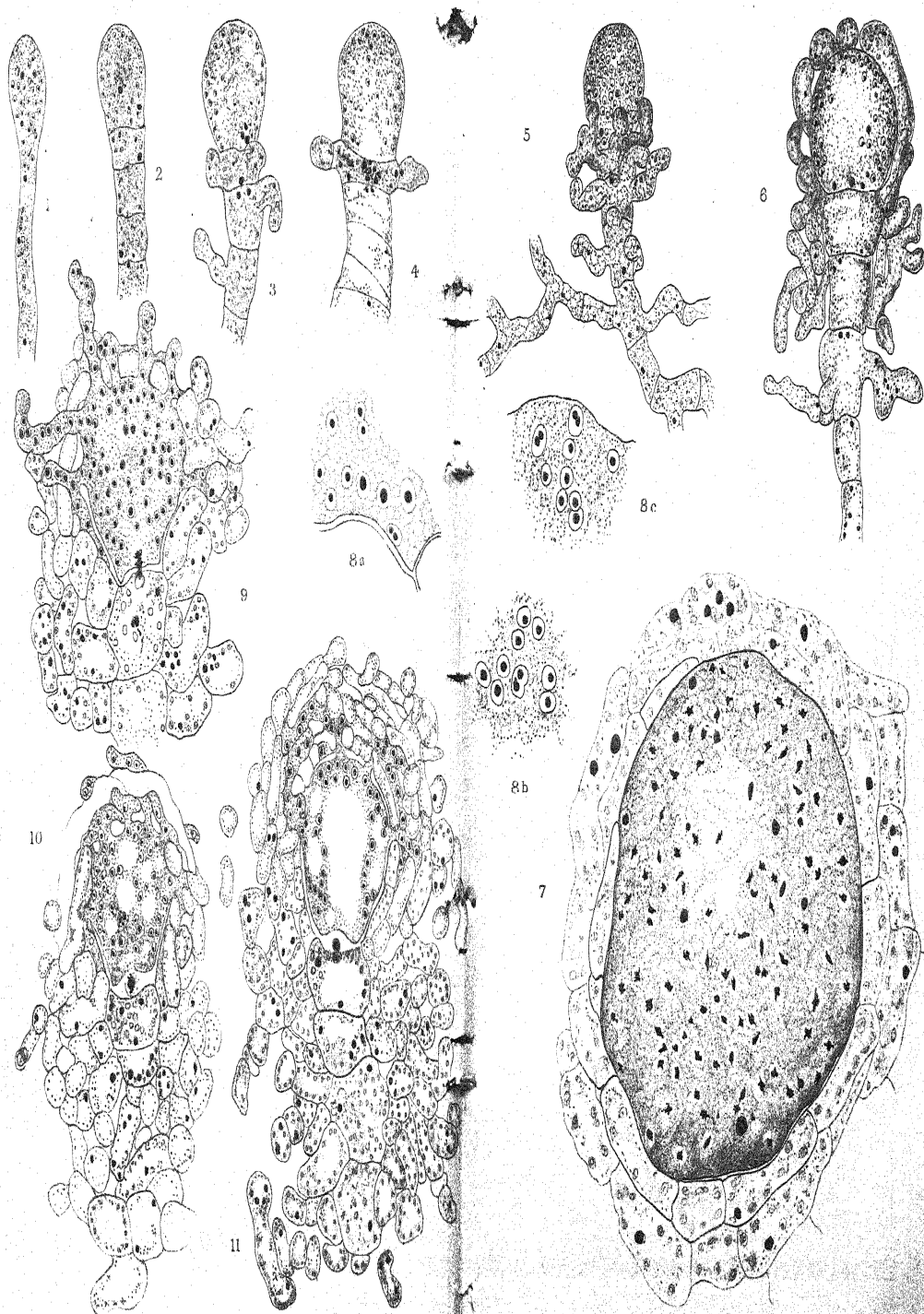
Figures 3 and 5 are drawn from more than one section.

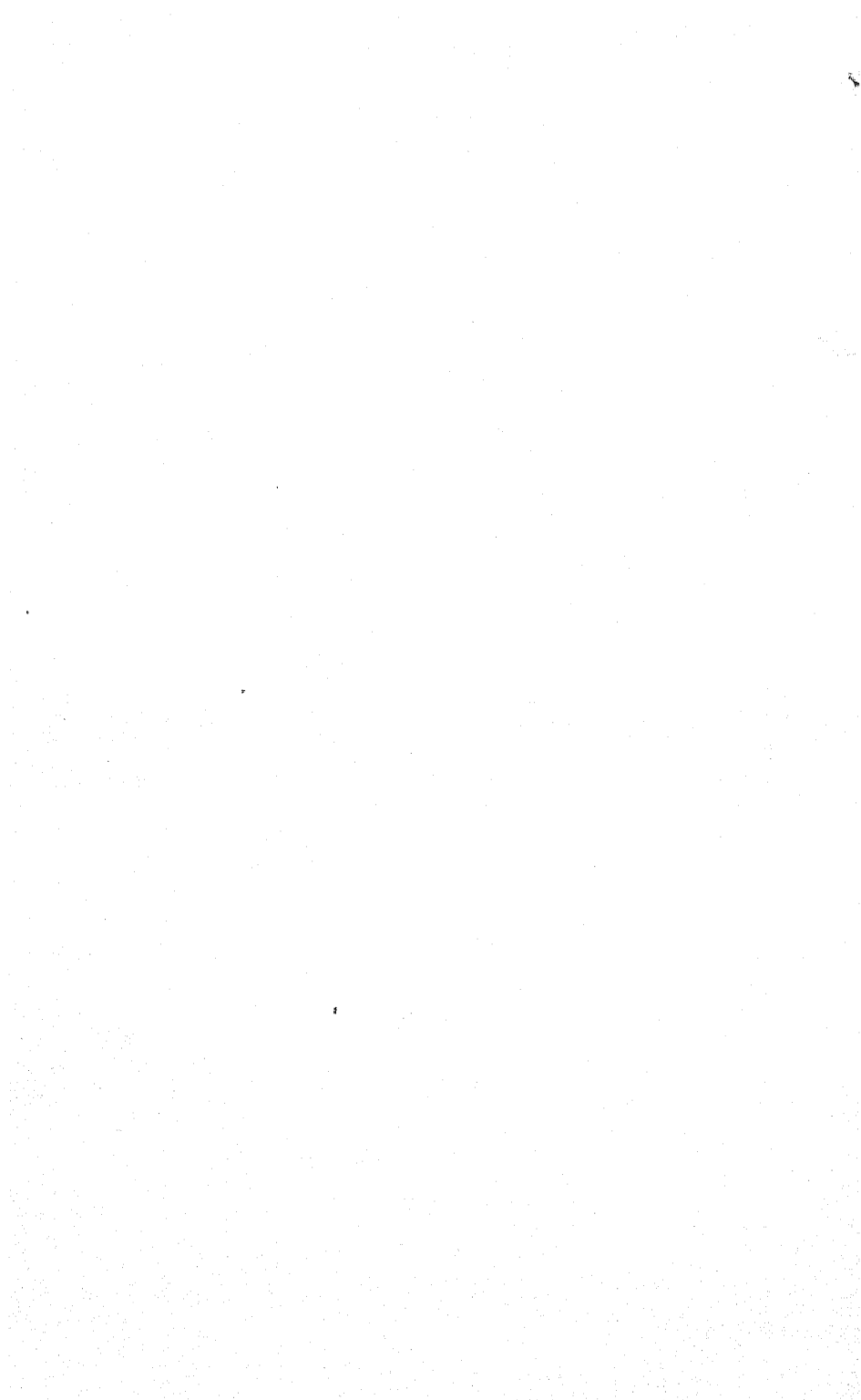
PLATE XIII.

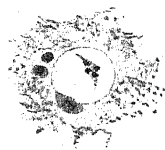
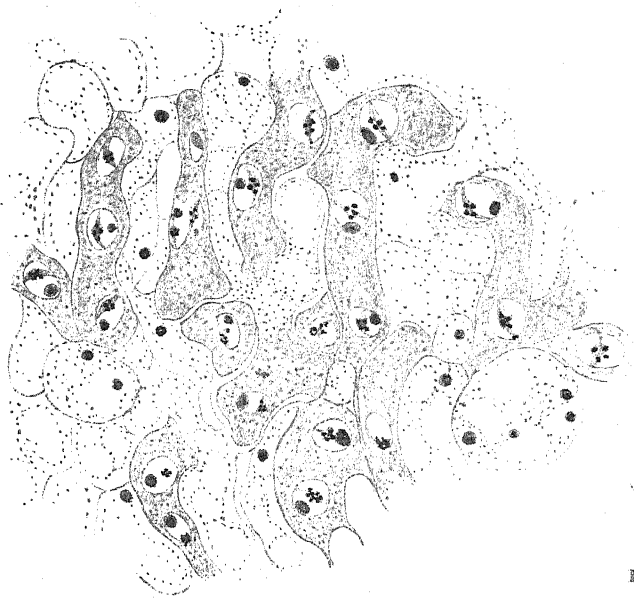
- Fig. 1. Very young aseptate archicarp. $\times 700$.
- Fig. 2. Young archicarp, with the oogonium just cut off. $\times 700$.
- Fig. 3. Rather older archicarp, the stalk branching to form the sheath. $\times 700$.
- Fig. 4. Archicarp of about the same age as that in Fig. 3, but showing signs of disintegration, especially in the empty stalk. $\times 700$.
- Fig. 5. Archicarp showing development of the sheath as a frill below the oogonium. $\times 700$.
- Fig. 6. Longitudinal section through a growing oogonium just overtopped by the cells of the sheath. $\times 700$.
- Fig. 7. Transverse section through an oogonium rather older than that in Fig. 6, showing all the nuclei in process of mitosis. $\times 1,600$.
- Fig. 8, *a*, *b*, and *c*. Stages of nuclear fusion in the oogonium. Fig. 8*a* shows nuclei of two sizes. $\times 1,600$.
- Fig. 9. Longitudinal section of oogonium showing young ascogenous hyphae. $\times 700$.
- Fig. 10. Similar stage in a rather smaller oogonium. $\times 700$.
- Fig. 11. Longitudinal section of older oogonium, showing ascogenous hyphae and several layers of sheath. $\times 700$.

PLATE XIV.

- Fig. 12. Mitosis in the ascogenous hyphae, nuclei in metaphase. $\times 1,600$.
- Fig. 13. A division in the oogonium from which the hyphae in Fig. 12 have arisen. $\times 2,600$.
- Fig. 14. Early anaphase in an ascogenous hypha from an ascocarp of about the same age as that in Figs. 12 and 13.
- Fig. 15. A division in the oogonium from which the hypha in Fig. 14 has arisen. $\times 2,600$.
- Fig. 16. Prophase of the meiotic division in the ascus showing eight gemini. $\times 2,600$.
- Fig. 17. A rather later stage of the same. $\times 1,600$.
- Fig. 18. Anaphase of the first division in the ascus, showing more than four chromosomes travelling to each pole. $\times 1,600$.
- Fig. 19. Metaphase of second division in the ascus, showing two nuclei with four chromatin masses each. $\times 1,600$.
- Fig. 20. Early telophase of third division in the ascus, showing distribution of four chromosomes to each ascospore nucleus. $\times 1,600$.





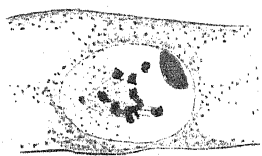


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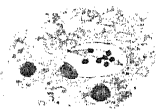
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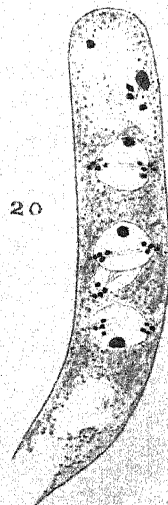
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Physiological Studies in Plant Nutrition.

II.¹ The Effect of Manurial Deficiency upon the Mechanical Strength of Barley Straw.²

BY

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With five Figures in the Text.

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INTRODUCTION.

IT was at one time believed that the strength of cereal straw, and therefore its resistance to 'lodging' by wind and rain, depended upon the silica content of the straw. It is, however, now realized that the supply of salts available to the plant and the atmospheric conditions of light and humidity under which it is grown are the factors controlling this property. For example, an excessive supply of nitrogen results in a sappy type of growth, the tillers being easily 'lodged'. Experiments at Rothamsted have shown that this effect is reduced by the addition of potassium salts, and that large doses of nitrogen can safely be given provided the nitrogen-potassium ratio is maintained at a normal level.

Several investigators have attempted to determine if the marked effects of varying manurial conditions are correlated with changes in the anatomical structure. They agree that large doses of nitrogenous manures

¹ Part I of this series appeared in this Journal, Vol. XL.

² Thesis submitted for M.Sc. degree of the University of London.

result in weakness, potassic and phosphatic fertilizers having the reverse effect; but no clear alterations in the anatomy were found to be caused by different manurings. The earlier investigators, Dassonville (1), and Kissel (2), obtained inconclusive results. Purvis (3) examined the effect of potash deficiency upon the anatomy of *Dactylis glomerata* from the Rothamsted grassland experiments. Although no measurements are given, she decided that no differences in the relative development of the various tissues were caused by varying the supply of potash. From measurements of the thickness of the cell-wall and the diameter of the lumen, it was shown that early in the season the ratio of diameter of lumen to thickness of cell-wall was increased by increase of potassium, but that this effect disappeared later. The conclusion was reached that the strengthening effect of potassium is not due to its effect upon the gross anatomy of the plant.

Advantage was taken of plants remaining from a large-scale experiment upon the effect of manurial deficiency on the physiology of barley plants to obtain further evidence on this subject.

EXPERIMENTAL METHODS.

The basis of the method employed was the measurement of the force required to crush portions of the straw of barley plants grown under varying manurial conditions, and the correlation of the strength thus found with the thickness of the tissues present. The barley employed was selected Plumage Archer, grown in the open in sand culture in glazed pots each holding 30 lb. of sand. The plants germinated on May 18, 1927, and were examined during the latter part of August and the first week of September. Four types of manuring were employed, one being 'complete' and the remainder being deficient in nitrogen, phosphorus, and potassium, respectively. The plants were grown three in a pot, each pot receiving the following weight (gram.) of salts.

	Na_2HPO_4 .	NaNO_3 .	K_2SO_4 .
Fully manured . . .	2.52	9.10	1.85
Nitrate deficient . . .	2.52	1.82	1.85
Phosphate deficient . . .	0.504	9.10	1.85
Potash deficient . . .	2.52	9.10	0.20

Each pot also received 0.37 gram. of CaCl_2 and 1.25 gram. of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

The plants were examined at approximately the same stage of their morphological history—just after the ears had 'shot'. Each plant was uprooted and placed in water in the laboratory. The large tillers in which the ear had 'shot' to a uniform extent were cut from the plant as required and the internodes numbered from above downwards, the peduncle being neglected. As a rule only five internodes of each tiller were large enough for experiment, the lower ones being very small and bearing numerous

adventitious roots upon the nodes. From each internode several portions of stem from 1.0 to 3.0 cm. in length were cut with a sharp scalpel, the majority being between 1.5 and 2.5 cm.; 1 cm. on each side of the node was neglected. At the same time thin sections were cut with a razor from varying positions in the internode and preserved in 50 per cent. alcohol.

Each portion of stem was placed at a constant distance from the fulcrum of two hinged plates, from the upper of which a brass rod projected. On this rod ran a small pulley-wheel by means of which a weight of any desired magnitude could be drawn slowly along the rod by a thread attached to the pulley. By this means the portion of stem was subjected continuously to a gradually increasing pressure. Crushing usually took place sharply. The distance of the pulley from the fulcrum when crushing occurred was then measured.

Then if:

S = the force in grammes required to crush 1 cm. length of tiller,

D = distance of the weight from the fulcrum in cm. when crushing occurred,

d = distance of the portion of the stem from the fulcrum in cm.,

W = weight in grammes moved along the beam, i. e. the weight of the pulley plus that of the suspended weight,

l = length of the portion of stem in cm., and if w = weight in grammes of the beam acting upon the stem,

$$S = \frac{WD}{dl} + \frac{w}{l}.$$

w was found by measuring the force required to just raise the rod by attaching a spring balance at the distance of d cm. from the fulcrum. By testing several portions of each internode, an average value of S for each internode of each tiller examined was found.

It was found desirable, when dealing with weak stems, to use a lighter apparatus; two hinged similar plates but without a rod upon the upper were therefore used, the weight being gently slid by hand over the upper plate until crushing occurred.

EXPERIMENTAL RESULTS.

The average force required to crush unit length of each internode of barley plants grown under varying manurial conditions is shown in Table I, the number of internodes upon which the values are based being shown in brackets. Those values which differ significantly from the corresponding values in fully manured plants are printed in black type. By the use of Fisher's (4) table of 't' it is possible, knowing the number in each sample and the variation within the samples, to estimate the probability that the observed differences between the means should occur by chance between

means of samples drawn from plants, which have been similarly treated. Where the probability against a difference of that magnitude occurring by chance is greater than one in twenty, the difference is accepted as 'significant'.

TABLE I.

Force in gramm. wt. required to crush 1 cm. length of straw.

Internode numbered from peduncle downward.	Fully Manured.	Nitrogen Deficient.	Phosphate Deficient.	Potassium Deficient.
1	229 (7)	241 (10)	151 (3)	231 (11)
2	347 (8)	445 (10)	306 (8)	508 (11)
3	465 (5)	786 (9)	572 (8)	764 (10)
4	771 (7)	1,604 (10)	1,000 (8)	813 (6)
5	1,407 (6)	2,543 (6)	2,097 (8)	698 (4)

The strength of the stem in the fully-manured plants increased rapidly from above downwards. The effect of nitrogen and phosphorus deficiency is to render this still more marked, as is shown by the large increase in the strength of the lower internodes, while the strength of the upper internodes approximates to the normal, i. e. to that of the fully-manured plants. Potassium-starved plants, however, show an interesting departure from this. The uppermost internode is normal but the middle internodes show a strength above normal. The fourth and fifth internodes are, however, weaker than the normal, the fifth internode being only half as strong as that of the fully-manured plants; the significance of this difference is greater than 100:1. In the cases of the fifth internode of the nitrogen and phosphorus-deficient series the chances are more than the 100:1 and 20:1 respectively against the differences from the fully-manured plants being due to chance. The difference between the third internode of the fully-manured and potassium-deficient series is high, but is prevented from being significant by an aberrant observation of 184 gramm. among the data on which the mean value of 764 is based. The data of Table I are shown graphically in Fig. 1. The belief of the practical agriculturist in the weakening effect of nitrogen and the strengthening effect of potassium is borne out by these figures.

It will be noted that the effect of mineral deficiency appears least in the upper internodes, becoming progressively more marked down the stem. The potassium-starved plants are weaker in the lowest internodes than in those just above, in sharp contrast to the other three types.

ANATOMICAL RELATIONSHIPS.

The anatomy of the barley stem follows the usual grass type. Beneath the epidermis occurs a zone of sclerenchyma, having a more or less wavy

outline internally. Embedded in this are small islands of unthickened chlorenchymatous cells. Between this and the schizogenous central cavity is a band of parenchyma, of which a variable amount abutting upon the sclerenchymatous zone possesses thickened and lignified cell-walls. Scattered throughout the tissues are the vascular bundles descending from

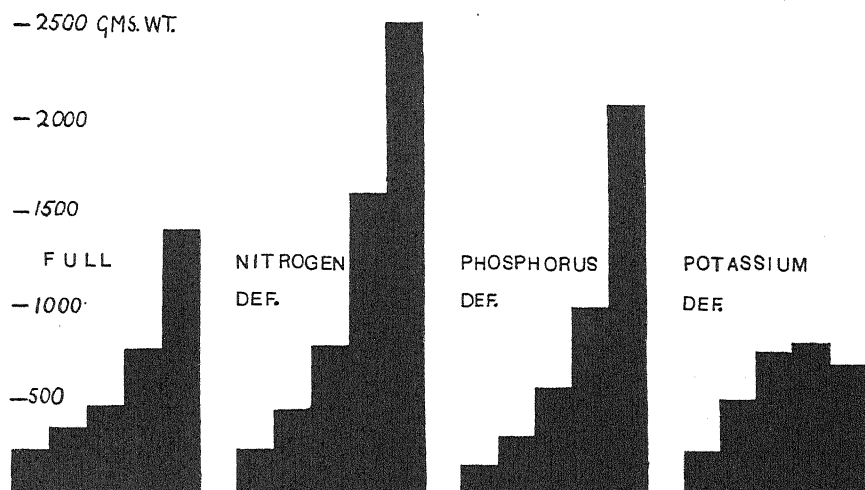


FIG. 1. The strength of succeeding internodes from above downwards.

the internode above and from the leaf attached at its base. The relative amounts of the tissues present in the stem were found by selecting at random two or three of the sections available for each internode; from these, drawings were made under a camera lucida and a low power of the microscope. The sections were stained with phloroglucin and hydrochloric acid, and the outline of the following four zones traced: (a) sclerenchyma, (b) lignified parenchyma, (c) unligified parenchyma, (d) the central lacuna. The epidermis and islets of chlorophyllous tissue were included in the sclerenchyma zone, while the lignified vascular elements in the unligified parenchyma were neglected.

It was found that considerable variation occurred in the depth of colour produced by phloroglucin, all tissues showing a pink coloration with phloroglucin and hydrochloric acid were accepted as lignified; it cannot be assumed, however, that the lignified tissues had all undergone lignification to the same extent. Nitrogen-deficient and phosphate-deficient plants alike showed lignification equal to that of fully-manured plants. Sections from potassium-deficient plants show considerably less depth of staining. All transitions were found from sections in which the sclerenchyma and vascular bundles were alone stained to sections indistinguishable

from those of fully-manured plants. The areas of the tissues were found with a planimeter, and the mean areas of the stem as a whole, of the three tissues, and of the central cavity in each internode were calculated. The use of mean values was considered permissible owing to the fact that variation between sections from the same internode is considerably less than that between sections from different internodes.

It was noted that even in cases of extreme reduction of the amount of tissue lignified in the potassium-starved plants, the vessels of the vascular bundles were always fully lignified.

Lignification consists of the incrustation and infiltration of the original cellulose cell-wall resulting in great changes in its physical properties. The unaltered cellulose wall is very elastic, but a comparatively small stress is sufficient to produce permanent distortion, but considerable extension can take place before fracture occurs. After lignification the tissue is far less extensible, and is capable of withstanding much greater stresses, but beyond the elastic limit fractures readily.

The thickness of the three zones under consideration appears to be markedly affected by the type of manuring. In Fig. 2 the thickness of sclerenchyma in each internode is shown graphically. It increases rapidly towards the base of the stem, but this increase is not proportionate to the increase in strength. For example, the strength of the fifth internode of the fully-manured plants is more than six times that of the first, whereas the thickness of sclerenchyma is only 2.4 times. The increasing strength in successive internodes from the apex cannot therefore be entirely ascribed to increase in thickness of sclerenchyma, and as, moreover, the lignified parenchyma shows only a slight increase in development, it would appear that the strength of these tissues must vary in the different internodes. The four diagrams in Fig. 2 show the same general form, but there are suggestive increases in the lower internodes of the phosphate- and nitrate-starved plants. These differences are not, however, significant. Reference to Fig. 3 shows that the thickness of the lignified parenchyma is much more uniform in all the internodes. Only in the case of the nitrogen-starved plants does the thickness increase steadily down the stem. There is, however, a striking reduction of lignified parenchyma in the potassium-starved plants. This failure to lignify the parenchyma results in a supernormal thickness of unligified parenchyma in the upper internodes as shown in Fig. 4. The large increase in the amount of unligified parenchyma is probably a partial explanation of the high value of the water-content of potassium-starved plants when compared with fully-manured plants. The almost constant breadth of the zone of the unligified parenchyma throughout the stem contrasts sharply with the remaining three types. The thickness is *significantly* increased above corresponding internodes of the fully manured in all the internodes of nitrogen-starved plants, in the third and

fourth internodes of the phosphate-starved plants and in all but the fifth of the potassium series.

The difference between the external and internal radii of the stem, i. e. the total thickness of the wall of the cylinder, is shown in Fig. 5. The

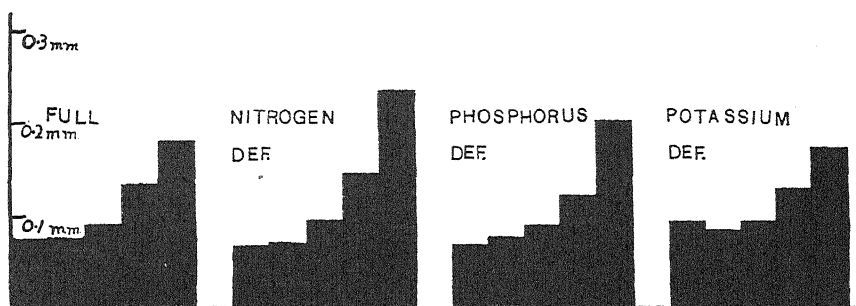


FIG. 2. The thickness of sclerenchyma in succeeding internodes from above downwards.

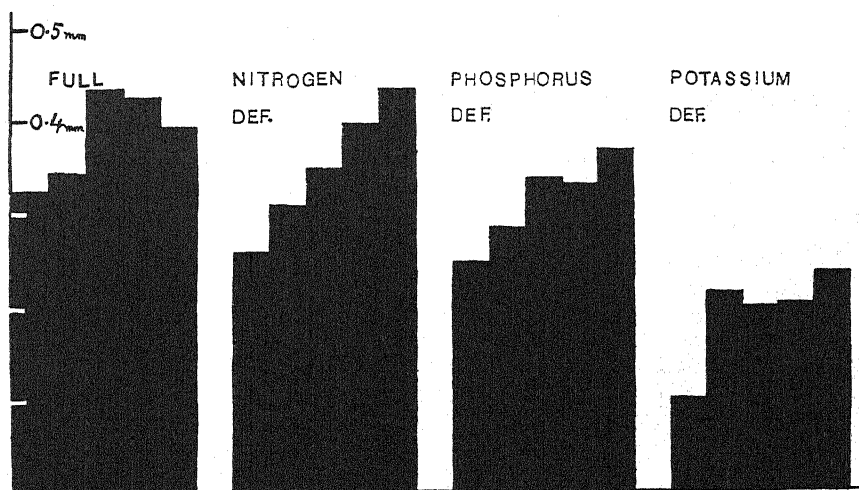


FIG. 3. The thickness of lignified parenchyma in succeeding internodes from above downwards.

thickness increases rapidly in the lower internodes; this is markedly affected by the manurial treatment. Nitrogen deficiency results in an increase in the thickness, a similar effect but not so clearly marked is to be observed in the case of phosphorus deficiency. With potassium deficiency the thickness is remarkably constant. Inasmuch as the diameter of the stem is in all cases reduced by mineral deficiency, a reduction in the diameter of the central cavity usually follows.

It was found that the ratio of the external diameter of the lignified tissue (i. e. the total diameter of the stem) to the internal diameter of the

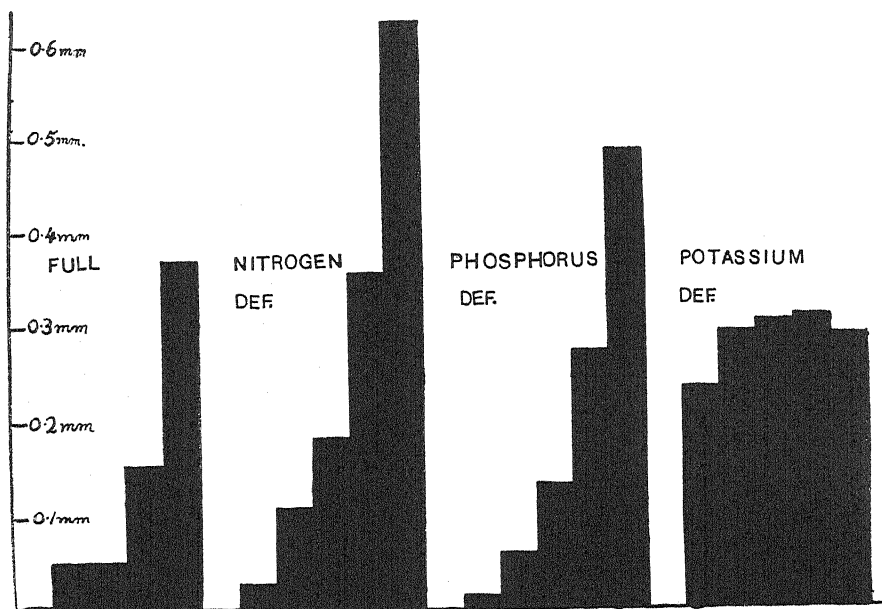


FIG. 4. The thickness of unligified parenchyma in succeeding internodes from above downwards.

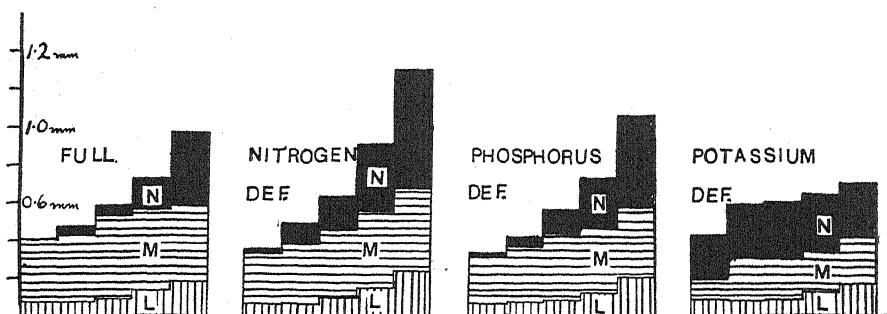


FIG. 5. The thickness of total tissue in succeeding internodes from above downwards, showing the relative proportions of the three tissues: L, sclerenchyma; M, lignified parenchyma; N, unligified parenchyma.

lignified tissue (sclerenchyma + lignified parenchyma) was constant for corresponding internodes with different manuring. The mean values for successive internodes are given in Table II, as well as the values for each manurial type.

TABLE II.

Internode number	1.	2.	3.	4.	5.
Fully manured	1.220	1.247	1.285	1.336	1.413
Nitrogen deficient	1.214	1.237	1.289	1.375	1.435
Phosphorus deficient	1.217	1.230	1.267	1.312	1.482
Potassium deficient	1.152	1.249	1.294	1.388	1.522
Mean	1.201	1.241	1.284	1.353	1.463

The value of 1.522 obtained for the fifth internode of the potassium-deficient plants is the only value differing significantly from those obtained for the fully-manured series. An analysis of variance showed that there was no significant manurial effect.

Thus the breadth of tissue that becomes lignified is directly proportional to the diameter of the internode. The existence of such a relationship renders it improbable that the number of parenchymatous cells that become lignified is dependent upon the amount of wall-forming materials available at the moment. The mechanical function of the sclerenchyma cells would seem to be pre-determined from the time they are laid down, and it appears that this is true of the cells of the lignified parenchyma also. This question is discussed later.

THE RELATION BETWEEN STRENGTH AND THE BREADTH OF THE TISSUES PRESENT.

The question arises, to what extent are the observed effects of manuring upon stem strength due either to alterations in the amounts of the tissues present, or to variations in the material composing them? It is probable that both these influences are at work, but the two cannot be directly separated owing to the fact that the strengths of individual tissues could not be estimated by the method used.

The relation between the strength of a hollow cylinder and the thickness of its walls, when strength is measured by the force required to crush it radially, is very nearly linear provided the material composing its walls is constant. The direct total and partial correlations between strength and the individual tissues have therefore been calculated and are given in Table III.

TABLE III.

	r ₁₂	r ₁₃	r ₁₄	r _{12.34}	r _{13.24}	r _{14.23}
Fully manured	0.854	0.340	0.945	0.485	0.246	0.825
Nitrogen deficient	0.912	0.654	0.910	0.234	0.813	0.909
Phosphorus deficient	0.740	0.563	0.859	0.410	0.486	0.644
Potassium deficient	0.235	0.478	0.074	0.053	0.611	0.471

1 = strength. 2 = thickness of sclerenchyma. 3 = thickness of lignified parenchyma.
4 = thickness of unligified parenchyma.

With the exception of the potassium-deficient series, highly significant and positive total correlations are seen in all cases. The elimination of the effect due to variations in thickness of the other two tissues results in a decided reduction of the correlation between strength and sclerenchyma. Thus the anomalous result is obtained of low values of the partial correlation between strength and sclerenchyma and high values for that between strength and unignified parenchyma. Reference to Fig. 5 shows that, of the three tissues, unignified parenchyma is by far the most variable. Furthermore, the correlation between thickness of sclerenchyma and of unignified parenchyma is high (except in the potassium-deficient series), as is shown in Table IV.

TABLE IV.

Fully manured . . .	+0.772
Nitrogen deficient . . .	+0.823
Phosphorus deficient . . .	+0.815
Potassium deficient . . .	+0.015

The high correlation between thickness of sclerenchyma and of unignified parenchyma suggests that other factors may influence the strength. One of these is the morphological status of the internode as is shown by the high positive values of the correlations, internode number and strength (*a*), and internode number and total tissue thickness (*b*), as show in Table V.

TABLE V.

	(<i>a</i>)	(<i>b</i>).
Fully manured . . .	+0.887	+0.878
Nitrogen deficient . . .	+0.936	+0.881
Phosphorus deficient . . .	+0.829	+0.864

It should be borne in mind that a high positive correlation between a variant and internode number indicates a high *negative* correlation with time, since the internodes have been arbitrarily numbered from above downwards.

It thus appears that the high value of the correlation of strength and unignified parenchyma ("14 Table III) arises from the fact that the unignified parenchyma is the most variable component of the total thickness of tissue (Fig. 5), and this variation is in the same direction as the increase of strength with internode number. When, therefore, the effect of thickness of unignified parenchyma is eliminated in the partial correlations of Table III, the effect of internode number is largely eliminated at the same time, leading to the anomaly noted above.

In the potassium-deficient series the correlation between strength and unignified parenchyma is greatly increased by the elimination of variance due to unignified parenchyma and to sclerenchyma. This is explained by

the value of the *negative* correlation between strength and sclerenchyma when age is eliminated, viz., 0.406 (significance of 100:1).

Attempts were made to obtain regression equations connecting strength, age, and the thickness of the mechanical tissues. With none of these was a good fit obtained. The equation $S = (f_1)l + (f_2)m + (f_3)n + C$ where l , m , and n are the thicknesses of the three tissues gave a good fit, but owing to the correlations of the tissue among themselves, the functions were difficult to interpret. It was observed, however, that the curve obtained when the mean strength of each internode was plotted against internode number did not differ significantly from an exponential curve in any case except in that of the potassium-deficient series. The linear regression equation between $\log_{10} S$ and internode number was therefore calculated. The equations are:

Fully manured:	$\log_{10} S = 0.19116x + 2.1433$
Nitrogen deficient:	$\log_{10} S = 0.22603x + 2.22143$
Phosphorus deficient:	$\log_{10} S = 0.27430x + 1.91061$

In Table VI the calculated and observed mean values of S for each internode are given.

TABLE VI.

Internode.	Fully Manured.		Nitrogen Deficient.		Phosphorus Deficient	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
1	229	216	241	280	151	153
2	360	335	445	472	306	288
3	465	521	786	793	572	541
4	771	809	1,604	1,335	1,000	1,018
5	1,407	1,256	2,543	2,247	2,097	1,915

The fact that the curve under discussion is an exponential curve demonstrates the fact that, within the limits of the error of the experiment, the strength of each internode bears a constant relation to the strength of the previous internode. The anti-logarithm of the coefficient of x in the above equation gives the relationship between strength of each internode and that of the internode next below it. These values, which may be said to characterize the effects of the manurial treatments on strength of straw, are as follows:

Fully manured	1.553
Nitrogen deficient	1.683
Phosphorus deficient	1.881

No rational expression was found that fitted the strengths found in the potassium-deficient series.

DISCUSSION.

The foregoing data provide the results upon which is based the general conclusion that the strength of the stem is decreased in the potassium-deficient series and increased in the nitrogen- and phosphorus-deficient series, and that these differences from the normal decrease as the stem is ascended.

The dependence of carbohydrate supply upon potassium content offers a possible explanation of these observations. Potassium deficiency has no influence upon the number of young tillers produced, whereas both nitrogen and phosphorus deficiency causes a considerable reduction in their numbers. The two latter series have available, therefore, a larger amount of potassium per plant organ than the fully-manured series. If potassium plays an essential part in the mechanical properties of the tissues or in their formation, this provides a possible explanation of those results. The continued decrease in the strength of succeeding internodes suggests the operation of some deficiency factor which increases with time. Extensive work upon the nutrition of barley plants in this Institute has led to the conclusion that growth proceeds until the concentration of salts within the plant has fallen to a definite level. This is of interest in view of the tendency of the upper internodes of all four series to approximate to the same strength. Gregory and Richards (5) have shown that the assimilation rate of potassium-starved plants shows a marked recovery to a supernormal rate after its initial fall, and suggest that this is connected with the supply of additional potassium from dying leaves and tillers. It is possible that some such recovery superimposed upon the age drift of strength accounts for the recovery to a supernormal value of the strength in the second and third internodes.

It has been demonstrated that there is a 'position' effect upon the strength of the tissues, the intrinsic strength as well as the thickness of the tissue increasing as the stem is descended. The change in the mechanical properties of the tissues is shown by the highly significant values found for the correlation between internode number and the ratio strength : thickness of total tissue. These are:

Fully manured	+0.867
Nitrogen deficient	+0.938
Phosphorus deficient	+0.729
Potassium deficient	+0.491

The decrease in strength of succeeding internodes in time may therefore be ascribed firstly to a decrease in the efficiency of the mechanical tissues and, secondly, to decrease in their amount.

It has also been shown that within the limits of experimental error the ratio of the external radius to the internal radius of the mechanical tissue is

constant for internodes of the same morphological age and is independent of manuring, except in the case of the lowest internode of the potassium-deficient series. This is of interest in view of the fact that the stems are laid down in embryonic form very early in the history of the plant, before internal manurial deficiency has occurred, except in the case of potassium-starved plants in which the incidence of internal starvation as evidenced by the sharp fall of assimilation rate is very early (5).

If cell size is proportional to the radius of the internode, the total number of mechanical cells in a cross-section of any internode of the same morphological status must be constant. This would indicate that the mechanical tissues are composed of elements whose function is determined irrespective of manurial deficiency. On the other hand, their mechanical strength is affected by the type of manuring given, the evidence pointing both to an effect upon the thickness of the tissue and upon its mechanical efficiency. The latter may be due either to alteration in the structure of the cell-wall or in its composition.

SUMMARY.

The paper deals with the results of an investigation of the effect of manurial deficiency upon the strength and anatomical structure of barley straw. The force in grm. weight required to crush 1 cm. length of stem radially is taken as a measure of strength.

The strength of succeeding internodes of fully-manured plants falls off rapidly. Nitrogen and phosphorus deficiency results in a large increase in the strength of the lower internodes, while potassium starvation decreases the strength of the lower and increases that of the middle internodes. The effects of manurial deficiency are most marked in the lower internodes, the upper ones approximating to the normal.

The variation in the thickness of the mechanical tissues follows that of strength but is not sufficient to account for the large differences observed. Total and partial linear correlations between strength and the morphological status of the internode, and between strength and the thickness of the three tissues, sclerenchyma, lignified parenchyma, and unlignified parenchyma, have been calculated. The conclusion is reached that the observed fall in strength of succeeding internodes is due both to decrease in the efficiency of the mechanical tissues and also to decrease in their actual amount.

Equations connecting strength and the morphological status of the internode are found to give a good fit in the fully manured, nitrogen-deficient and phosphate-deficient series. The relation between strength and internode number is logarithmic, the strength of each internode being a constant fraction of that next below, within the limits of the error of the experiment, the value of the fraction being dependent on the type of manuring applied.

The ratio of the external radius to the internal radius of the mechanical tissues is found to be constant for internodes of the same status independent of manuring. It appears that the mechanical function of the elements composing them is determined at a very early stage.

It is suggested that the observed effects of mineral deficiency are explicable on the assumption that potassium is essential to the production of an efficient mechanical tissue.

The work reported here was carried out at Rothamsted at the suggestion of Dr. F. G. Gregory, and the author's thanks are due to Sir John Russell for granting the necessary facilities. The author desires to thank Professor V. H. Blackman and Dr. F. G. Gregory for stimulating advice and criticism.

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Notes on Conifers.

III. Further Points in the Morphology of *Larix europaea* DC.

BY

W. T. SAXTON.

With seven Figures in the Text.

WHEN the writer published some notes on the morphology of *Larix* (8) a few months ago, nearly all the observations recorded below had already been made, but publication was delayed in order to confirm during

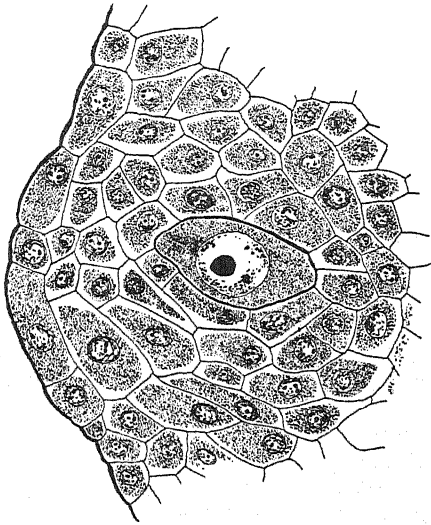


FIG. 1. Median horizontal section of very young ovule of *Larix europaea*, showing the megaspore-mother-cell. Oct. 31, 1928. $\times 500$.

the autumn of 1929 what already appeared probable from preparations made a year earlier.

In 1879 Strasburger (9) gave an account of the early development of *Larix europaea* based on material collected in March and April, 1878. He states that the female cone passes the winter with the young ovule in the spore-mother-cell stage, which is undoubtedly the case, and that the spore-

mother-cell arises by the divisions of a hypodermal archesporial cell. This last statement is apparently based on the youngest stage figured by him, in which the integument has only just begun to develop and in which the spore-mother-cell is separated from the epidermis by an axial row of three small cells supposed to be derived from a primary wall cell. During the

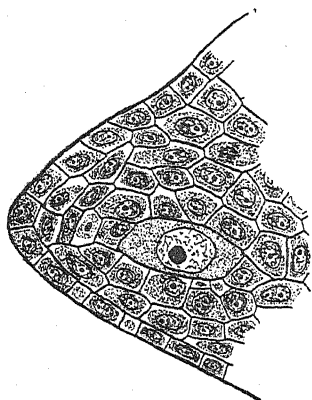


FIG. 2.

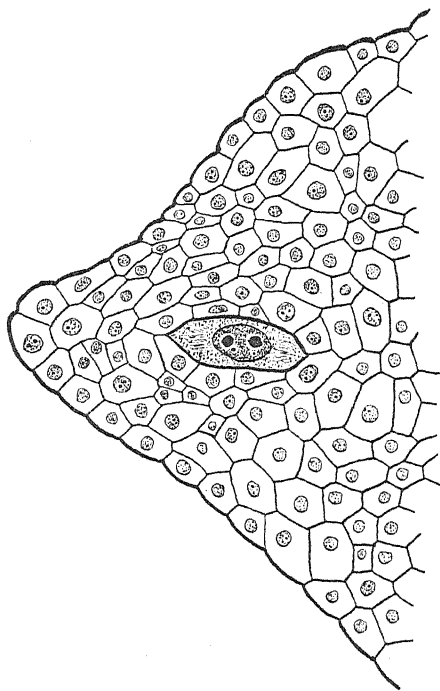


FIG. 3.

FIGS. 2 and 3. Median vertical sections of slightly older ovules, showing the megaspore-mother-cell. Jan. 19, 1928. $\times 365$.

last three winters a number of young Larch cones have been examined at intervals, during January, February, and September to December, 1928, and January to March, and September, 1929. Out of a large number of ovules no case has been found where the arrangement of cells closely resembles Strasburger's figures. The three median sections which appeared most similar are reproduced in Figs. 1-3. In Figs. 1 and 3 there is no indication whatever of an axial row of cells suggesting derivation from a hypodermal archesporial cell; Fig. 2 is the only example seen, out of a large number, in which the cells above the mother-cell seem, at first sight, to form a fairly definite axial row; but there are four such cells, not three, and the third cell only just touches the plane of the section, while the two above are cut medianly, so that the appearance in the longitudinal plane perpendicular to this would not be the same. Even in this case, therefore, the appearance of

an axial row is illusive. In the early spring the spore-mother-cell is very conspicuous, but by examining successively earlier stages it was found impossible to identify it before about mid-September. In cones collected on September 24, 1928, the spore-mother-cell could still be recognized, but only with difficulty, while in two cones collected on September 11, 1929,

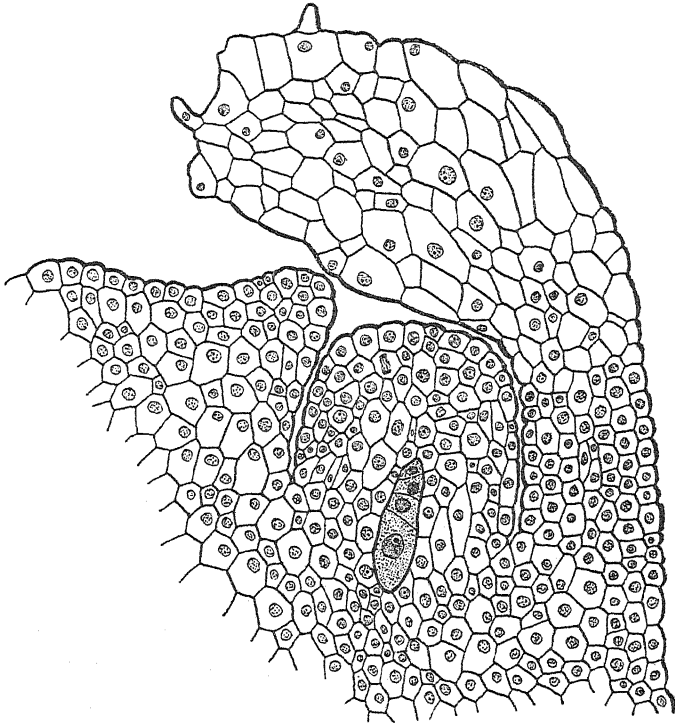


FIG. 4. Median vertical section of an older ovule ready for pollination, showing the stigmatic flap and a row of four megaspores. Feb. 19, 1928. $\times 215$.

the most careful search failed to reveal the slightest trace of such cells, or of any cell which could be interpreted as a hypodermal archesporium. The conclusion seems inevitable that the spore-mother-cell is directly picked out from an undifferentiated nucellus early in the autumn, and not derived from a recognizable hypodermal archesporium. In practically all other conifers spore-mother-cells are picked out directly from among the deeper cells of the nucellus, the only other exception recorded being *Taxus*. Here also Strasburger (loc. cit.) inferred the presence of a hypodermal archesporium on similar evidence, and Dupler (3) has recently described and figured such an archesporial cell. It is not clear, however, either from the text or the figure, whether the cell so named was visibly differentiated from the surrounding cells or not, so that the actual occurrence of a hypodermal archesporium in *Taxus* still requires confirmation.

In the Larch, at a slightly later stage, a unilateral stigmatic flap makes its appearance, a fact recorded and figured by Gélénznoff (4), Baillon (1), Strasburger (9), Goebel (5), and others, and probably well known to the earlier students of Conifer morphology, though obviously overlooked by Lawson (7) in his account of *Pseudotsuga*. Attention has recently been

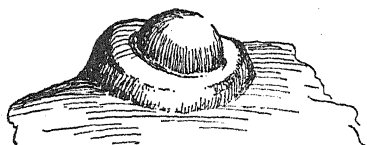


FIG. 5.

FIG. 5. View of very young ovule when the integument is only represented by a scarcely perceptible rim. March 10, 1929. (Corresponds to about Feb. 10, 1928). $\times 100$.



FIG. 6.

FIG. 6. View of a somewhat older ovule, when the stigmatic flap has begun to appear. March 23, 1929. $\times 100$.

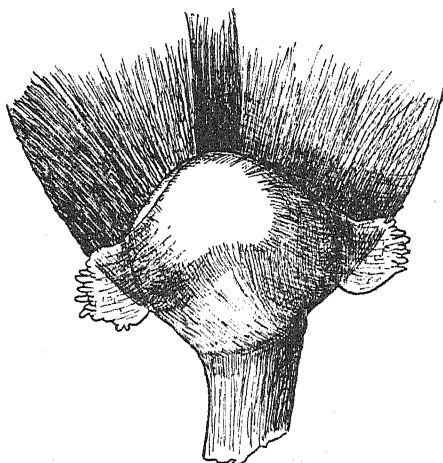


FIG. 7. View of an ovuliferous scale with two ovules very slightly older than Fig. 4. March 25, 1929. (Corresponds to Feb. 19, 1928; cf. Fig. 4.) $\times 25$.

again directed to this curious feature by Doyle (2), and it is illustrated in Figs. 4-7. Fig. 4 shows the appearance of the flap in median longitudinal section, while Figs. 5-7 show the very young ovule as seen in the living state under the dissecting microscope during the early development of the integument. They show much more clearly than sections the relations of the ovule and integument to the ovuliferous scale.

Strasburger (loc. cit.) states that after the first division of the spore-mother-cell the upper cell divides again, but not the lower, the latter becoming the functional megaspore. This account is not correct. Either

three or four megaspores are formed in an axial row, but it is the *upper* of the two cells formed by the first division which may fail to divide again. Fig. 4 shows the structure of an ovule in which four megaspores have been formed. Juel (6) has given details of these stages in *Larix sibirica*, which agree with those here recorded for *L. europaea*.

It may be of interest, in view of the great climatic difference between the winters of 1927-8 and 1928-9, to compare the dates of similar stages in those years. The stage shown in Fig. 4 was met with on February 19, 1928, but not till about five weeks later in 1929. On the other hand no such difference was found in the male cone, in which spore-mother-cells were found dividing on approximately the same dates each year. During February, 1929, male cones were collected and examined every four hours during the twenty-four and every day for about a fortnight or more. Divisions were found in progress at all times, but were never simultaneous in a loculus, as they are in some Conifers such as *Pinus* and *Taxus*. Even during the extreme cold of the second week in February dividing spore-mother-cells seemed as frequent as at any other time.

Thanks are due to Miss L. E. Hawker, who made the drawings for Fig. 1 and Figs. 3-7.

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Notes on Conifers.

IV. Some Points in the Leaf Anatomy of *Fokienia Hodginsii* Henry and Thomas and *Libocedrus macrolepis* B. and H.

BY

W. T. SAXTON.

With five Figures in the Text.

MANY members of the Cupressaceae, as is well known, and a few other Conifers, have a very specialized shoot system in which the branchlets are more or less horizontal and are flattened in the horizontal plane. The leaves in such Cupressaceae are decussate, one pair placed laterally on the edges of the flattened stem and the other pair appressed to the upper and lower surfaces respectively. The shoot itself is more or less jointed, each 'joint' bearing the two pairs of leaves just mentioned, the lateral pair below the facial but the internode between them exceedingly short. The lateral leaves of such shoots are usually flattened in the radial (antero-posterior) plane, i. e. perpendicular to the dorsiventral plane of normal leaves, while the facial leaves are flattened in the usual way. Both lateral and facial leaves are markedly decurrent, being fused with the stem for the greater part of their length with only the tips free.

Among various scattered references to the anatomy of this dorsiventral type of shoot, Frank (5 and 6) studied that of *Thuja occidentalis* with especial reference to the distribution of the stomata. He also tried the effect of turning the shoot over, so that what was originally the lower surface became the upper; after this had been done he found that there was a gradual change in the tissue, so that at the end of the growth period the original upper surface had acquired the structure of the lower, or nearly so. He also noticed that the dorsiventral shoot is frequently found in a vertical position, when the side to the outside of the bush has the structure of the upper side of horizontally flattened twigs. From this he concluded that the difference in structure must be a phototropic and not a geotropic reaction.

Klemm (8) describes and figures the branchlet structure in several species, two of the most striking being *Thujaopsis dolobrata*, S. and Z., and

Thuja gigantea, Nuttall (*T. plicata*, D. Don.), both of which show considerable resemblance to the *Libocedrus* described below. An earlier but much less complete account was given by Lazarski (9) in 1880, but little stress is there laid on the special type of branchlet now under discussion. More recently Prause (10) has given a fairly extensive comparative account of the leaf anatomy in the Cupressaceae, in which he devotes a good deal of attention to the special features of dorsiventral shoots. He deals, amongst other genera and species, with *Thuja*, *Thujopsis*, *Cupressus*, *Libocedrus decurrens*, and *Tetraclinis articulata*. He states that the lateral leaves are boat-shaped on the edge of the stem, implying some sort of comparison with the equitant leaves of many Iridaceae, and Daguillon (3) describes the lateral leaves of *Thuja* and *Biota* as equitant, but such a boat-shaped form is not found in the two species here described, nor does it seem to have been characteristic of those studied by Klemm. Prause (10) describes the thickenings which are found where the facial and lateral leaves overlap, and which prevent the leaves from being too closely appressed to one another, thus facilitating the passage of air to the stomata; in *Tetraclinis* these thickenings are said to become hair-like and to fit together in a tooth-like manner. He further draws attention to the distribution of the stomata and resin-canals in the species studied. Transfusion tissue is always developed, and special attention is given to this tissue in the earlier paper by Lazarski (loc. cit.).

Radially flattened leaves are rare in other families of Conifers, but attention was drawn to their occurrence in young plants of *Podocarpus dacrydioides* by Griffin (7) and in *Acropyle Pancheri* in a paper by Sahni (11), in which he gives figures and an explanatory diagram to indicate the peculiarities of the radially flattened type of leaf. He also indicates the probable occurrence of the same leaf-type in certain species of *Podocarpus*.

The material upon which the present account is based consisted of dorsiventral shoots of *Libocedrus macrolepis*, B. and H., and of the juvenile form of *Fokienia Hodginsii*, Henry and Thomas, both obtained from the Temperate House at Kew in June, 1929, by kind permission of the Director. Only young plants of *Fokienia* were available; the leaves and branchlets get gradually smaller in the older tree and it is possible that the anatomy may also differ. The general morphology of the jointed branches is exactly of the type already described, and in both species the four leaves of one 'joint' all have a different structure. The radially flattened leaf on one side only differs from that on the other in being its looking-glass image (no such difference is usually found between the leaves of a pair in other plants), but the upper leaf of the facial pair has palisade, with little or no spongy tissue and no stomata, while that on the lower side has stomata and spongy parenchyma and no palisade.

Vegetative shoots of *Libocedrus macrolepis* and of the juvenile form of *Fokienia* are extremely alike in appearance, and the points of difference

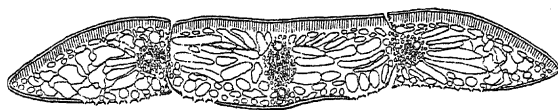


FIG. 1.

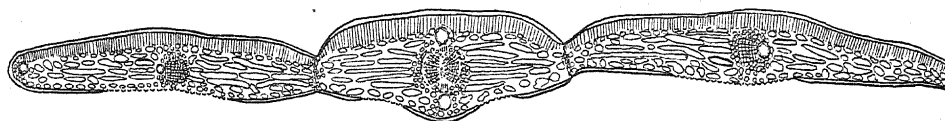


FIG. 2.

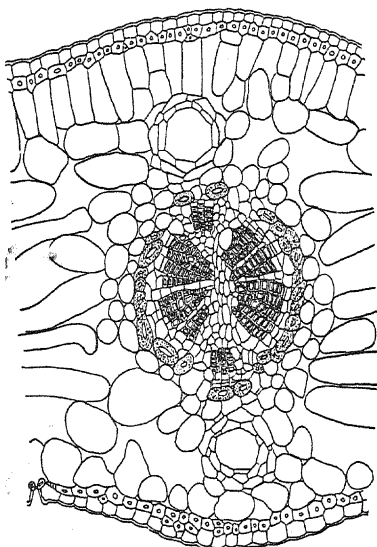


FIG. 3.

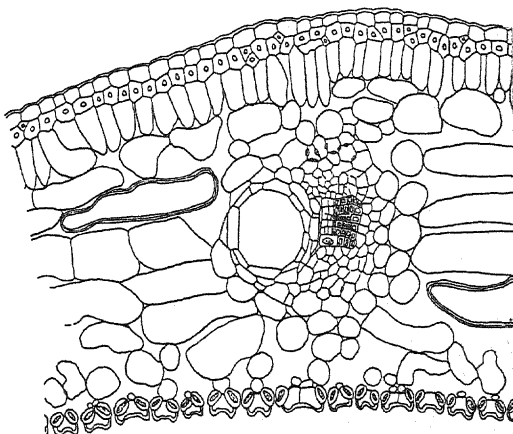


FIG. 4.

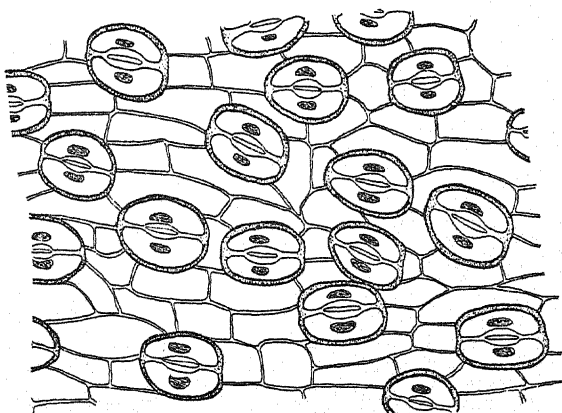


FIG. 5.

FIGS. 1-5. 1. Transverse section of a branchlet of *Libocedrus macrolepis*. $\times 25$. 2. Similar section of a branchlet of *Fokienia hodginsii*. $\times 24$. 3. Central part of the preceding, showing detail. $\times 145$. 4. Part of left-hand leaf of Fig. 2, showing detail. $\times 145$. 5. Stomata on lower epidermis of *Fokienia hodginsii*. $\times 290$.

between the adult forms, mentioned by FitzPatrick (4) in a recent paper, hardly apply to the juvenile condition. It may be observed, however, that the vein of the lateral leaf lies very close to the branchlet in *Libocedrus*, but runs almost medianly through the young leaf of *Fokienia*. This difference can be seen much more clearly in section. The points mentioned, and other features in the structure, are indicated in the figures. Fig. 1 is a diagrammatic sketch of the structure seen in a transverse section of a horizontal branchlet of *Libocedrus macrolepis*. The lateral leaves are marked off sharply from the stem by a line of smaller cells and by fairly deep dorsal and ventral grooves, but there is no visible line of demarcation between the stem tissue and the facial leaves. Palisade occurs opposite the whole upper surface of the branchlet, and spongy parenchyma opposite the lower. The stomata lie along two bands, each of which lies astride one of the grooves on the lower surface, and they are also found in both dorsal and ventral grooves. It seems evident that the description of the under surface of the foliage given by FitzPatrick (4), as having 'stomatic depressions, white patches sunk in hollows in the leaves', is not borne out by these sections, either in this species or in *Fokienia*, nor can any such depression be seen in the living plants. Possibly the description was based on dried specimens, in which shrinkage of the more delicate tissue below the stomata might easily bring about such an appearance.

The vascular strands of the lateral leaves in *Libocedrus* are seen to be closely adjacent to the junction of stem and leaf, in contrast with *Fokienia*, as already mentioned.

Fokienia Hodginsii shows the phenomena of the dorsiventral branchlet in a more striking manner than any of the Cupressaceae previously studied. Fig. 2 is a diagrammatic drawing to about the same scale as Fig. 1 to facilitate comparison with *Libocedrus*. The most striking difference is in the position of the vascular strands. The stomata are here found in bands covering the central part of the lower side of each lateral leaf, and two smaller bands are seen on the surface of the lower facial leaf. As grooves are absent there are no other stomata. The transversely elongated cells of the 'accessory transfusion tissue' (Worsdell (3)) or 'Hydrostéréome transversale' (Bernard (1)) are extremely well developed in both species. Figs. 3 and 4 show the detail of the central parts of the branchlet (3) and of the left-hand lateral leaf (4) respectively in Fig. 2. There is a continuous band of hypoderm both above and below except opposite the stomata, and the latter are very closely crowded; a single resin canal is found on the phloem side of each leaf-trace; the vascular cylinder of the stem is closely surrounded by bast fibres, but these die out when the strands pass out into the leaves. Transfusion tracheides, on the other hand, make their appearance as soon as the leaf-trace is quite free from the central cylinder, and seem to be slightly better developed on the upper side of the lateral leaves. A few

of the cells of the accessory transfusion tissue become thick-walled, the stratification of the wall being very clearly shown. The closely crowded arrangement of the stomata in *Fokienia* is even more strikingly shown in surface view, as seen in a drawing of a small part of the epidermis (Fig. 5). It would perhaps be difficult to find any plant in which the stomata cover such a high proportion of the total area over which they extend. The number per square millimetre is between 300 and 350, by no means an exceptional figure for stomata in general but very high in relation to the rather large size of the stomata in this case, and much higher than those usually recorded for Conifers (Czech (2), Frank (5 and 6), Strübing (12)).

Thanks are due to Dr. Hill for his kind permission to obtain the material, and to Mr. Raffill, in charge of the Temperate House, Kew, for his help in procuring it.

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Studies of the Physiological Importance of the Mineral Elements in Plants.

I. The Relation of Potassium to the Properties and Functions of the Leaf.

BY

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With six Figures in the Text.

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I. INTRODUCTION.

IT has long been known that an adequate supply of potassium is necessary for the photosynthesis of starch, and since the element has not been found to occur in starch or any other important product of carbon assimilation, there has been much speculation concerning its part in the process. That its role is important is shown by the wide range of plants in which its presence is known to be necessary. Starch formation has been shown to be facilitated by potassium in such diverse cases as the algae, the protenemata of mosses (Reed, 16), sunflower seedlings (Briggs, 5), and the potato plant (Maskell, 14).

The last species affords peculiar advantages for the study of this problem for two reasons. As is well known to all growers, its potassium requirements are unusually high, in correlation, perhaps, with its exaggerated formation of starch. This is likely to help the experimental handling of the problem, especially under field conditions, where delicate control cannot be established, and also when quantitative analyses are required. In the second place, the plant segregates its abundant reserve carbohydrate in well-defined storage tubers, thus enabling it to be estimated with ease.

Taking advantage of these facts, Maskell (14) has made an investigation with potato plants growing under normal agricultural conditions. The only unusual manipulation was the approximate control of the potash content of the soil, which was varied on neighbouring plots. On land supplied with potassium in the form of sulphate, the quantity of starch formed per acre was in each case higher than when no potassium was added. This was partly accounted for by an increased weight of tubers, and partly by a larger percentage of starch in the dried material. Maskell found that correlated with this greater starch formation there was, with potassium sulphate, a rise both in the rate of starch formation in the leaves, and in the rate of its translocation from them. There remains the problem of how these accelerations are brought about. To help in solving this it seemed desirable to discover what further effects, if any, potassium has upon the functions of the leaf, and during the summer of 1926 a number of observations were carried out towards this end. Data were collected bearing upon the starch formation, translocation, number, area, weight, water content, and senescence of potato leaves.

2. LEAF NUMBER.

Other things being equal, an increase of leaf area should lead to an increase in the total quantity of starch formed by any individual plant. It is therefore of interest to discover whether additions of available potassium lead to such an increase of surface, but it is unfortunately very difficult with the potato plant to obtain a reliable estimate of the leaf area. A normal adult plant of the available variety (Kerr's Pink) may produce as many as two hundred leaves, with an average of seven leaflets each. It is, moreover, necessary in comparison between two sets of plants to take a large number of individuals, since variation is high.

In the season 1927 an attempt was made, with the variety Arran Comrade, which produces a less luxuriant growth than Kerr's Pink, to estimate the average leaf area of plants receiving high and low supplies of available potassium. Weekly samples were taken from the time the leaves appeared above the ground, both the high and low potassium samples containing ten plants, one being selected at random from each of ten plots.

All leaflets were stripped from the plants taken, and photographed on blue print paper as quickly as possible. The prints were planimeted at leisure, and the total areas summed. It was proposed to continue this sampling throughout the life-cycle of the plant, but after the first few samples the number of leaves had become so great that the photographic printing could not be carried out with the assistance available, and the experiment had to be brought to a close. Up to this point the results indicated were as follows:

TABLE I.

Average Leaf Area of Ten Potato Plants in sq. cm.

	Available Potassium.	
	Low.	High.
July 6	30.01	67.96
13	71.62	281.93
20	385.92	1217.25

An experiment carried out on August 3 with five plants in each sample indicated a reversal in the relative areas, which was accompanied by a corresponding reversal in dry weights. Thereafter dry weight measurements, which were continued throughout the growing period, indicated very slight differences between the two groups, which were not statistically significant, since variation was high and the samples small.

In the absence of reliable results from direct measurements a second method is of interest. An approximate indication of leaf area, when dealing with a reasonably large sample, is afforded by the total number of leaves. This crude estimate may be improved by measuring the area of a selected leaf or leaflet. Knowing the average number of leaves produced, and the average area of a selected leaflet, some idea may be formed of the relative leaf areas of the plants on different plots. Measurements of this kind were carried out on adult material (var. Kerr's Pink) in the season 1926.

TABLE II.

Plot totals: Number of Leaves on Twenty Plants.

	No Potassium added.	Potash Manure Salts.	Potassium Chloride.	Potassium Sulphate.
Series 1. Aug. 12	2419	2023	2305	1980
Series 2. Aug. 15	2231	1965	2488	2543
Series 3. Aug. 18	3098	2193	2606	2748
Treatment Totals	7748	6181	7399	7271
Mean	2582.67	2060.33	2466.33	2423.67

To estimate the leaf number, observations were made on 1/50 acre plots, each containing about 300 plants. Twenty plants were taken at

random on each plot, and the total number of leaves, irrespective of their condition, counted on each plant. Four series of counts were made, each involving four plots. In the first three series, the plots had received respective dressings of potassium chloride, potassium sulphate, 'potash manure salts' (a low-grade fertilizer), and no additional potassium, besides an otherwise normal potato manuring. The results of these counts are summarized in Table II.

There appeared to be a reduction in the number of leaves produced by plants receiving additional potassium, and the figures were therefore examined by Fisher's Analysis of Variance method (11). It was assumed that the difference of occasion had no effect upon the leaf number, since the dates were not far removed from one another. The variance due to individual plot effects could then be separated from the treatment effects, and the following values were obtained:

TABLE III.

Analysis of Variance of Leaf Number.

	Sums of Squares.	Degrees of Freedom.	Variance.	Log _e Variance.	Found. Z	For $P = 0.05$.
Treatment	457736	3	152579	11.9355	0.7798	1.0953
Plot	494954	2	247477	12.4193		
Remainder	311431	6	51905	10.8572		
Total	1264121	11				

The 'remainder variance' represents the variance due to differential responses of the various plots to different manurial treatments. The variance in leaf number due to treatment is greater than this remainder variance, and it is necessary to ascertain whether the excess could arise from any chance combination of manures and plots, or whether it is great enough to indicate a significant effect of treatment upon leaf number independent of the differences in plots. A result is considered significant when it could not arise by chance combinations more than once in twenty times ($P = 0.05$). The criterion developed by Fisher for this comparison is half the difference between the natural logarithms of the two variances ($= Z$). The value of Z required for significance is dependent upon the number of degrees of freedom available for estimating the variances, and in the present instance is $= 1.0953$. The value $\frac{1}{2} (11.9355 - 10.8572) = 0.7798$, and therefore fails to reach significance.

The number of leaves counted on the P.M.S. plots was considerably lower than on the others, and although in the general analysis this difference is obscured by the similarity between other treatments, there remains the possibility that the mean P.M.S. value is significantly lower than, say, the mean value for no additional potassium. The difference between these

mean values was therefore examined by means of their standard error (S).

As an estimate of this quantity the value $\sqrt{\frac{\text{Remainder variance} \times 2}{\text{number of replications}}}$ was taken, and since the available samples were small the further quantity

$$t = \frac{\text{P.M.S. mean} - \text{no K mean}}{S}$$

was also determined. The value of t required for any given degree of probability is dependent upon the number of degrees of freedom (n) available for its estimation. Tables of t for varying probabilities and values of n are given by Fisher (11). For $P = 0.05$ with 6 degrees of freedom the value is 2.447, and as calculated above the several comparisons give:

No K — P.M.S. 2.805; KCl — P.M.S. 2.183; K_2SO_4 — P.M.S. 1.952. The average number of leaves produced on P.M.S. plots is therefore significantly lower than on plots without added potassium, but there is no significant difference between the means when P.M.S. is compared with other treatments. Neither are the differences between the addition and absence of KCl or K_2SO_4 significant since they are even less than that of the previous comparisons.

In the fourth series of counts the case of K_2SO_4 was examined further. A different arrangement of plots was employed, in which the sulphate was withheld or added to the extent of 1, 2, or 4 cwt. per acre. Counts were carried out on each of the four plots in the manner described for series 1-3. The total number of leaves on each plot, twenty plants being counted, were as follows:

TABLE IV.

Series 4.

Potassium sulphate added in cwt. per acre.	Leaves counted.
0	2,124
1	2,017
2	1,682
4	1,760

Analysis of Variance of Leaf Number.

	Sums of Squares.	Degrees of Freedom.	Variance.	Log _e Variance.	Found. Z	For $P = 0.05$.
Treatment	6545.8	3	2181.9	7.6881	0.3663	0.4787
Remainder	79797.6	76	1049.9	6.9566		
Total	86343.4	79				

In the comparison between treatment and remainder variances the significant value of Z is here 0.4787. Half the difference between the

natural logarithms as calculated = 0.3663, and the treatment variance is therefore not great enough to be significant. An inspection of the values at the head of Table IV will show, however, that there remains the possibility of a significant difference between 'high' and 'low' treatments (2 and 4 compared with 0 and 1).

Treatment variance may therefore be further analysed as follows:

TABLE V.

	Sums of Squares.	Degrees of Freedom.	Variance.	Log _e Variance.	Z Found.	For $P = 0.05$
High—(2 and 4) with low— (0 and 1)	6107.5	1	6107.5	8.7174	0.8804	0.6729
Other com- parisons	438.3	2	219.2			

For a comparison between these variances and the remainder variance the significant limit of $Z = 0.6729$. Nearly all the treatment variance is accounted for by the comparison between high and low treatments which gives a value for Z of 0.8804. The plots receiving high potassium sulphate thus show a significant reduction in the number of leaves produced, when compared with little or no addition.

3. THE AREA OF A SELECTED LEAFLET.

The leaflet chosen for this estimation was the penultimate pinna of the fourth leaf counting from the apex of any haulm. No differentiation was made between left and right pinnae, but to avoid the correlation known to exist between opposite pinnae of the same leaf (14) samples were taken singly and never in opposite pairs. The material to be measured was obtained from a field experiment of sixteen plots, twelve of which were also sampled in making the leaf counts of series 1-3. The experiment contained four treatments, each repeated four times. The plots were arranged as a four by four 'latin square', each treatment occurring once, and only once, in each row across, and in each column up and down the experiment. With this limitation they were placed at random within the square. The treatments, as previously stated, were, no additional potassium, potassium chloride, potassium sulphate, and potash manure salts.

Leaflets were collected for measurement every fourth day from July 12, when the plants had already developed a large number of leaves, to September 10, when the leaflets were turning yellow. Each sample consisted of six leaflets per plot taken from different plants at random.

A rack containing sixteen stoppered weighing bottles was taken on to the field, and each sample of leaves enclosed, as it was picked, in a bottle numbered to correspond with the plot. The small quantity of air enclosed

by the bottles was soon saturated with water vapour and further transpiration by the leaflets thus prevented. This was important, as potato leaves shrink markedly with loss of moisture (see p. 183). When sampling was completed the rack was taken to a field laboratory, where the leaflets were

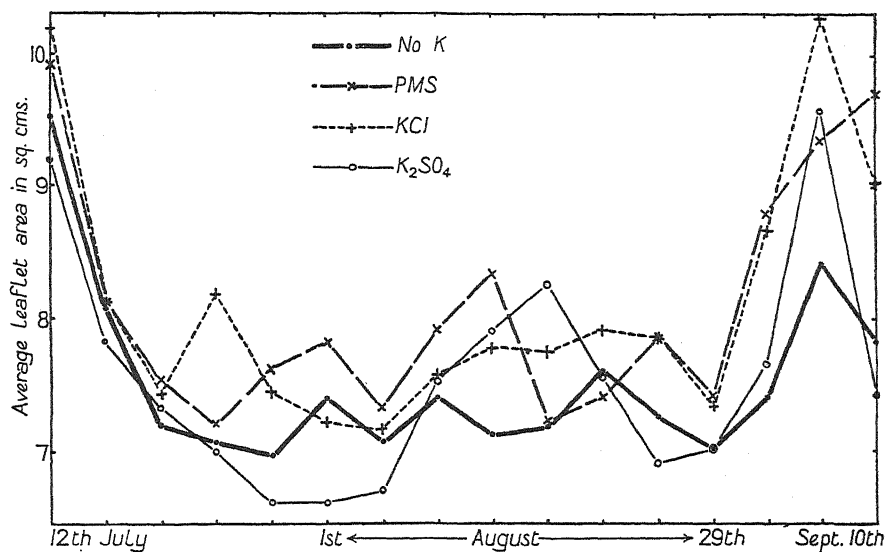


FIG. 1.

taken from the bottles and photographed on blue print paper, and the areas of the prints afterwards measured with a planimeter. The mean treatment values of the twenty-four leaflets on each occasion are shown in Fig. 1.

An analysis of the figures gave the following results:

TABLE VI.
Analysis of Variance of Leaf Area.

	Sums of Squares.	Degrees of Freedom.	Variance.	Loge Variance.	Found. Z	For $P = 0.05$.
Treatment	17.90187	3	5.96729	1.7863	1.0146	0.7798
Plot { Rows	29.04102	3	9.68034			
Columns	1.37800	3	0.45933			
Remainder	4.70660	6	0.78443	1.7571		
Occasion	153.39477	15	10.22632	2.3253	1.2841	
Differentials	284.40521	225				
Total	490.82747	255				

Owing to the planning of the experiment it is possible to compare similar plots both across and up and down the area. The variation between like plots in the transverse direction is given in the above analysis by 'Rows' and the variation travelling at right angles to this by 'Columns'. The two

added together give the total variance ascribable to plot differences. The remainder variance is the random variance due to chance combinations of plots and treatments without reference to occasion, and that labelled 'differential', the third degree differential variance due to chance combinations of occasion, plot, and treatment. As a basis for comparison in tests of significance the 'remainder' variance 0.78443 is used. The values of

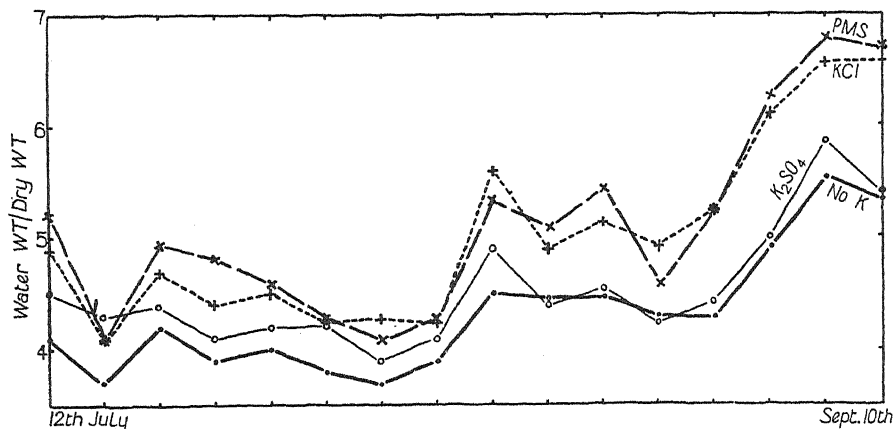


FIG. 2.

Z found and the values required for significance ($P = 0.05$) are given in Table VI, and show that the variance due to treatment, rows, and occasion are all significant. The columns variance, however, is not significant.

The mean areas of leaflets given by the different treatments were:

No potassium 7.590; K₂SO₄ 7.578; KCl 8.112; and P.M.S. 8.124 sq. cm. It is, therefore, evident that the treatment variance is almost entirely accounted for by the comparison between no added potassium + K₂SO₄ on the one hand, and KCl + P.M.S. on the other. There is practically no difference between K₂SO₄ and absence of added potassium. The KCl and potash manure salts are alike in containing a rather high percentage of chlorine, which is absent in the other treatments. The correct interpretation, therefore, of the significant treatment variance is, probably, to ascribe the increase in area which it denotes to the action of chlorine rather than that of potassium. This view receives further support from the results of the following section which suggest a possible mechanism for the changes.

4. WATER CONTENT.

It was shown by de Vries (10) that potassium is associated with organic acids in the cell sap of various tissues, including leaves, and he suggested that the power of the vacuoles to absorb water is due in part to the dissolved

potassium. With a view to investigating the problem in its relation to leaves, the water content of a large number of leaflets was examined. The material was that also used in leaf area measurements, the collection and initial handling of which has already been described on p. 178. Immediately on arriving at the field laboratory the leaflets were weighed in their respective bottles without further handling. No attempt was made to secure individual weighings of the leaflets, as these were not wanted. After being photographed for the area determinations, the leaflets were returned to the weighing bottles and put to dry in an oven at approximately 100° C. After twenty-four hours the bottles were transferred to a desiccator and, when cold, stoppered and weighed. The difference between the first and final weighings was taken as the total quantity of water in the leaflets. The moisture content of the leaves was expressed as water weight / dry weight (see Fig. 2), and the variance of these results analysed by the method already employed, the following values being obtained :

TABLE VII.

Analysis of Variance of Water Content per Lot of Six Leaflets.

	Sums of Squares.	Degrees of Freedom.	Variance.	Loge Variance.	Z Found.	For $P = 0.05$.
Treatment	29.848792	3	9.949597	2.2975	1.8240	0.7798
Plot { Rows	1.312332	3	0.437444			
Columns	0.168023	3	0.056008			
Remainder	1.554752	6	0.259125	2.6495		
Occasion	110.827712	15	7.388514	1.9999	1.6752	0.6931—0.6729
Differentials	23.861432	225				
Totals	167.573043	255				

The treatment variance is clearly significant. The weighing for the individual treatments gave the following average values:

No potassium 4.328; P.M.S. 5.139; KCl 5.035; K_2SO_4 4.509.

These figures fall readily into two groups; P.M.S. and potassium chloride treatments giving high water contents; no potassium and potassium sulphate giving relatively low water contents. The first two are alike in containing chlorine, and the total treatment variance can therefore be further analysed.

TABLE VIII.

	Sums of Squares.	Degrees of Freedom.	Variance
Cl—no Cl	28.4755	1	28.4755
Other comparisons . . .	1.3733	2	0.6767
All treatments as in Table 7	29.8488	3	

Practically the entire treatment variance is therefore due to the chlorine comparison, and there is no possibility of a significant potassium effect on

the water weight / dry weight ratio. The foregoing results, therefore, do nothing to support the view that the osmotic pressure in leaf cells is due to the presence of dissolved potassium.

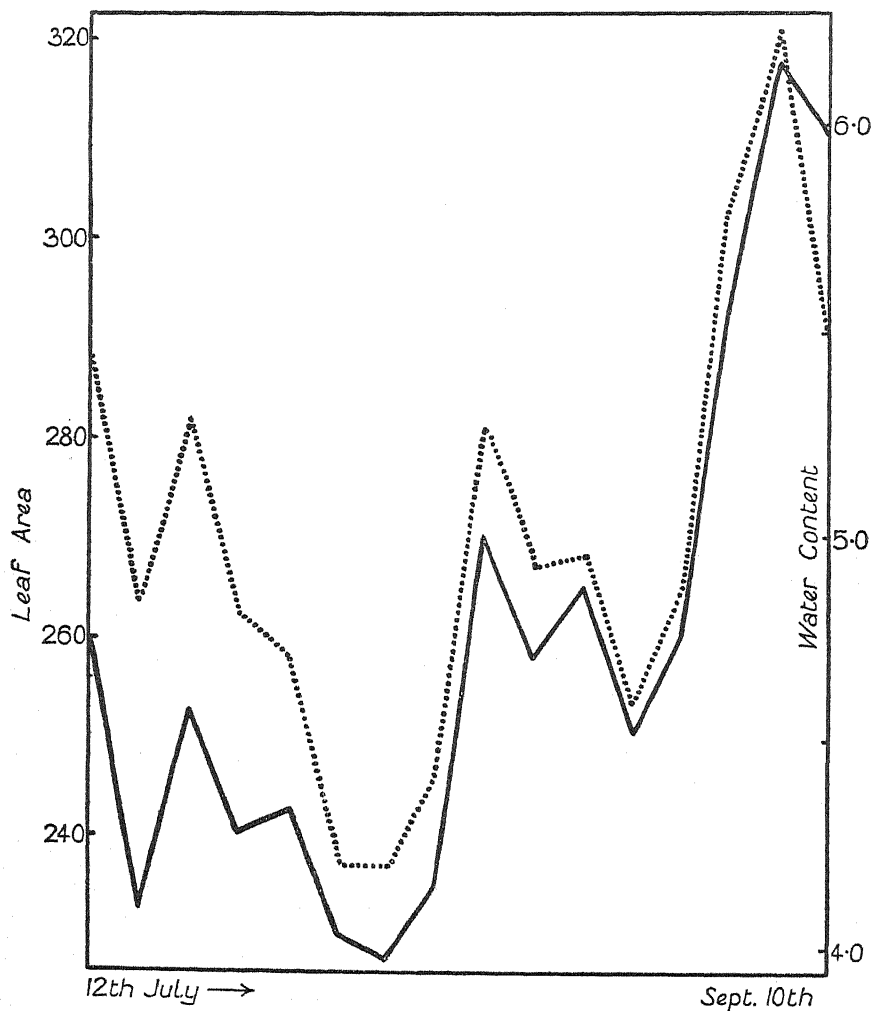


FIG. 3. Water content per gm. of dry weight is shown by the continuous line; leaf area (sq. cm.) per gm. of dry weight by the dotted line.

In addition to the treatment variance there was a large and significant variance due to 'occasion', the water content varying, that is to say, on the different sampling occasions, even on identical plots. These variations were no doubt dependent upon the complicated soil and atmospheric conditions which control transpiration, and a detailed analysis is beyond the scope of the present paper. An interesting correlation was observed, however,

between water content and area, both of which had been observed, on the same series of leaflets. To improve the comparison, areas were calculated, like the water contents, in terms of unit dry weight. A similar result might have been obtained by giving the weights of water in absolute quantities per leaflet. Fig. 3 indicates the very close nature of the correlation.

The fluctuations in leaf area cover as much as 25 per cent. of the mean value, and although changes of water content do not necessarily account for all this they must be the cause of a very high proportion. It follows, therefore, that water content, and the factors controlling it, must be taken into careful account in estimations of the leaf area of such species as the potato. In the foregoing analysis of leaf area measurements, these effects are included under 'occasion', and the estimate of treatment variance is therefore not disturbed by them. In experiments where periodic measurements of leaf area are used as an index of growth, the question seems to require more attention than it has received. Similar fluctuations of leaf area, depending upon external controlling conditions, have been observed with sunflower plants by Thoday (18).

5. LEAF WEIGHT.

In the observations on leaf water content just recorded, incidental measurements were obtained of the weights of the leaflets after drying. As these weights are also of interest in relation to other properties of the leaf, they were themselves examined by the analysis of variance method. The principal figures are given in the following table :

TABLE IX.

Analysis of Variance of Dry Weight of Leaflets.

	Sums of Squares.	Degrees of Freedom.	Variance.	Loge Variance.	Found. Z	For $P = 0.05$.
Treatment	2339	3	779.7			
Plot { Rows	13458	3	4486.0	8.4088	0.8756	0.7798
{ Columns	845	3	281.7			
Remainder	4672	6	778.7	6.6577		
Occasion	29440	15	1962.6	7.5822	0.4623	0.69—0.67
Differentials	147554	225	655.8			
Total	198308	255				

In this analysis the variances due to treatment, columns, and differentials, are obviously not significant since they are actually less than the remainder variance or scarcely exceed it. Of the other two, 'occasion' also proves to be non-significant, indicating that the leaflets had attained much the same weight at whatever stage of the life-cycle they were sampled. There was not, for instance, any marked tendency for leaflets produced late in the season to show a more restricted growth than those produced early,

nor could one expect to find a correlation between their development and the weather. The large time variance in area shown in Table VI cannot, moreover, be accounted for by variations in the quantity of dry material, and its dependence on water content is thus confirmed.

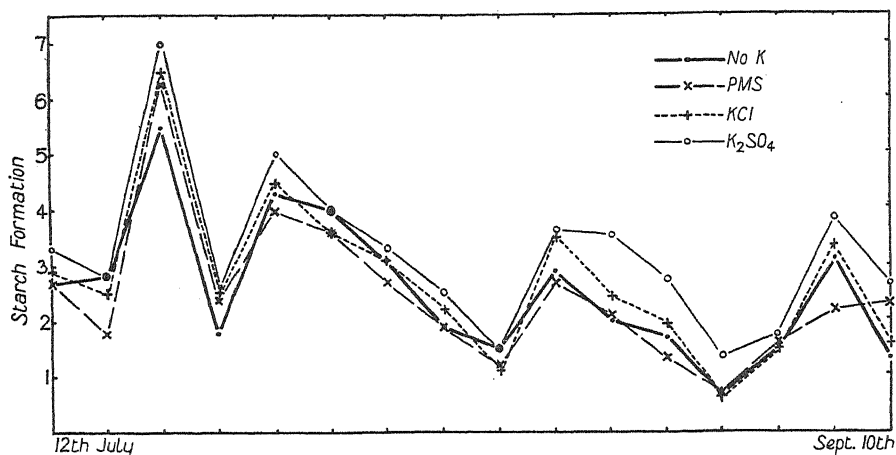


FIG. 4. Starch content, in terms of tone scale numbers, for 4-day periods, from July 12 to Sept. 10.

6. STARCH FORMATION AND TRANSLOCATION.

Reference has already been made (p. 174) to the observations of Maskell (14) on the effect of potassium manuring upon the rates of formation and removal of starch. His experiments were carried out in the summer of 1925 with the potato variety, Kerr's Pink. Four plots, receiving respectively no additional potassium, low grade potash manure salts (P.M.S.), potassium chloride, and potassium sulphate, were sampled on eleven occasions. An extension of this experiment was carried out by the present author in the following season. Instead of using single plots for each treatment, sampling was carried out over a four by four latin square, containing the same treatments, each treatment being replicated four times. The samples were taken every fourth day from an early stage in growth up to a period when yellowing was well advanced, covering sixteen occasions in all. The material was similar to that taken for leaf area and water content estimations, i. e. a penultimate pinna was removed from each of six leaves, the leaves being always the fourth counting from the apex; the plants and haulms from which they were derived were taken at random from the plot. Only green leaflets of healthy appearance were taken, the experiment being stopped when leaflets at the selected position began to turn yellow.

The method of estimating starch formation was that employed by Maskell, with minor alterations. The selected leaflets, together with their

opposite pinnae, were covered with black paper envelopes on the evening prior to their removal from the plant. At one o'clock on the following day one envelope of each pair was removed and the leaflet, by now destarched, was exposed for two hours. At three o'clock the leaflets were picked in pairs, decolorized with alcohol, and immersed in a strong solution of alcoholic iodine diluted with an equal quantity of water. The depth of the colour produced was judged in each case against a black-violet colour scale, Ridgway 59^{''''} (17). The scale contained nine tones, and, with practice, judgement could be made to half a tone. The difference between the tone numbers of the exposed and covered leaflets was taken as a measure of the starch formation during the standard two hours' exposure, and the mean of the six numbers as the representative value for the plot. The treatment values for each occasion are shown graphically in Fig. 4, and analysis of the results yielded the following data:

TABLE X.

Analysis of Variance of Starch Formation.

	Sums of Squares.	Degrees of Freedom.	Variance.	Log _e Variance.	Found.	Z For $P = 0.05$.
Treatment	20.798	3	6.933	1.9363	1.9441	0.7798
Plot { Rows	9.324	3	3.108	1.1339	1.5429	0.7798
Columns	1.799	3	0.600	1.4892	0.7206	0.7798
Remainder	0.840	6	0.142	2.0481		
Occasion	425.705	15	28.380	3.3457	2.6488	0.6931
Differentials :						
Time × Treatment	18.707	45	0.418	1.1277	0.5398	0.6499
Time × Rows	34.151	45				
Time × Columns	17.951	45				
Remainder	23.588	90				
Totals	552.863	255				

The significant variances are shown by heavy type. A large variation is ascribable to treatment, and it is therefore of interest to discover to which of the comparisons it is due.

The mean values for starch formation were No K 2.55; P.M.S. 2.47; KCl 2.72; K₂SO₄ 3.51; and it is therefore clear that the bulk of the treatment variation is accounted for by the comparisons between potassium sulphate and the remaining three treatments. To examine the significance of these differences the quantity

$$t = \frac{M_1 - M_2}{s}$$

was calculated as in the case of leaf number. The values obtained were K₂SO₄—No K 4.424; K₂SO₄—P.M.S. 4.729; K₂SO₄—KCl 3.640; KCl—No K 0.6383. Reference to Fisher's tables shows that as six degrees of freedom are available for the estimation of s , the differences

between sulphate and other treatments could arise by chance less than once in twenty times. The difference caused by adding KCl on the other hand, does not indicate a definite increase. The small decrease shown by the P.M.S. mean is certainly not significant, and the large treatment variance is, therefore, almost entirely due to the beneficial effect of potassium sulphate on the rate of starch formation.

The differential variances were further analysed. Interest centres upon the varying effects of treatment at different times, since if such a variance were found significant the data would be worth further investigation in regard to the correlation between manurial conditions on the one hand, and weather or ageing on the other. As shown in Table X, however, this variance (Time \times Treatment), just failed to reach a significant value. This may well be due to lack of differentiation between the treatments containing chlorides, masking the effects produced by the sulphate, and the curves of Fig. 4 do suggest that the effect of K_2SO_4 is greatest in the later part of the season. The correlation between ageing and treatment is investigated further in the following section.

In this experiment the period during which the leaflets were darkened was so long that the starch was almost entirely removed from them. It was not possible, therefore, to analyse the tone numbers of the covered leaflets for a rate of removal of starch as was done by Maskell. This method is furthermore open to the objection that since the quantity of starch formed in light is dependent upon treatment, the amount remaining after a period of darkness need not be proportional to the rate of its removal, but will also be influenced by the different amounts initially present. Thus, since potassium sulphate increases the amount of starch formed during illumination, its effect, if any, in accelerating translocation will tend to be masked. As it was desirable, however, to obtain some information on this point, a special experiment was carried out midway through the season. Sampling was similar to that in the starch formation experiment. On each occasion six leaflets from the selected position on the plant were taken from each of the sixteen plots, and removed in a bottle, numbered to correspond with the plot. The leaflets were not covered or manipulated in any way before picking, so that on decolorizing and treating with iodine a measure was obtained of their starch content under natural conditions. Samples were taken at every third hour from 10 a.m. on July 27, to 10 a.m. the following day. On July 27 the sky was overcast and total darkness set in at 10 p.m., lasting until 4.15 next morning. During this period the collection of the leaflets was carried out with the help of an electric torch, the illumination from which could have no appreciable effect on the starch content of the leaflets. A number of leaves of the required position had previously been marked with white tabs to facilitate this work. After each collection the material was carried to a field

laboratory and immersed at once in alcohol, the starch determinations being carried out subsequently at leisure. Average values from twenty-four determinations were thus obtained for each treatment on each occasion. The results are shown graphically in Fig. 5. From 1 p.m. to 10 p.m. on

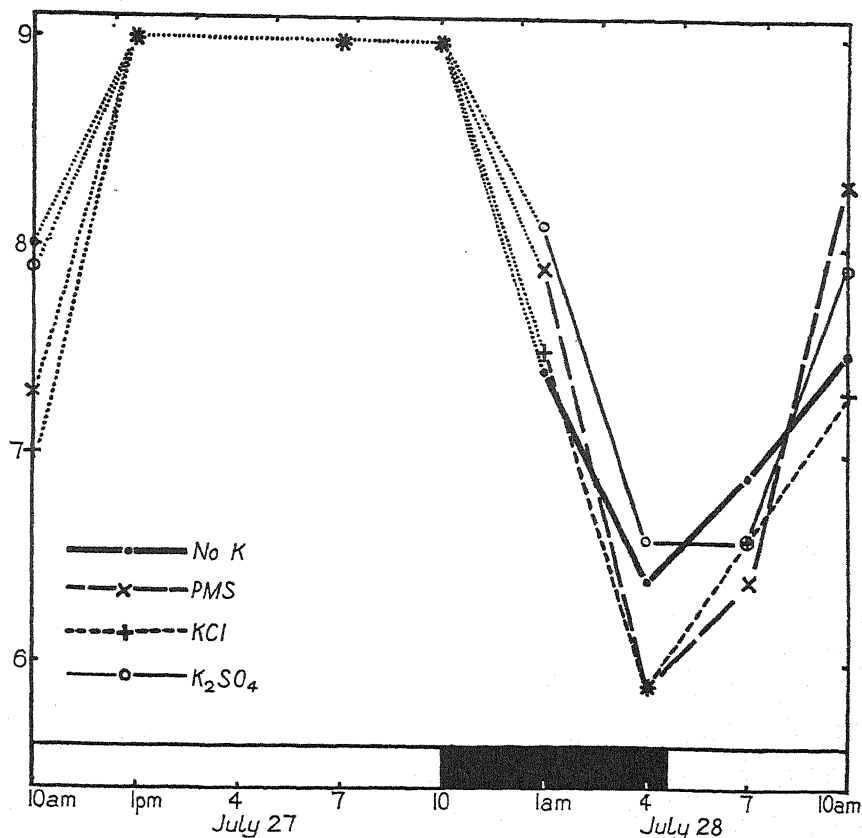


FIG. 5. Starch content, in terms of tone scale numbers, during 24 hours.

the 27th, the treated samples gave intense black colours, their starch contents being, in consequence, beyond the range of the determination. From 10 p.m. to 4 a.m. there was a steady reduction in the quantity of starch in the leaves of every treatment series, and from 4 a.m. onwards a rise. During the whole of the time from 10 p.m. until 10 a.m., during which the method could be usefully applied, distinct values were obtained for the different treatments. The most important point is that the leaflets receiving potassium treatments show a relatively high starch content in the afternoon and evening, and that during the night their curves cut that of the no potassium treatment, presumably owing to the faster rate at which starch is removed from them. The daytime samples, in which the colour was

invariably fully black, were discarded and the variance of the remaining values analysed to see whether the indicated differences between the treatments were significant. Owing to the relatively small number of sampling occasions, viz., five, the treatment variance failed to reach significance, and it could not, therefore, be expected that the time \times treatment differential variance, indicating differences in the rate of starch removal, would be significant. Neither was the largest of the differences between two treatment means great enough to indicate a certain result. Further analysis was therefore abandoned, and the results of the experiment must be regarded as suggestive rather than conclusive.

7. COLOUR.

As a first attempt to examine the effects of potassium upon ageing, the most obvious attribute of the leaf was considered. In the early part of July a change of colour first became apparent, at which time it was limited to the lower leaves, the middle and upper remaining a fresh green until a much later period. The original colour differed slightly on different plots, and a practised eye could judge the manurial treatment from the massed colour of the plot as seen from a small distance. On the experiment previously referred to, for instance, the P.M.S. plots had an obviously lighter tone than the potassium sulphate plots, and in an adjacent experiment involving nitrogenous manures, the colour differences were much more obvious, the presence of abundant nitrogen being correlated with a lush greenness.

The latter series of plots, referred to in future as the 'Quantitative Experiment' was of the 'window pane' variety. It contained four equal blocks, each of which had sixteen plots within it. The treatments it was designed to investigate were increasing doses of potassium and ammonium sulphates. Application was made at the rate of 0, 1, 2, or 4 cwt. per acre, and there was a plot in each block for each possible combination of the two fertilizers. Within the blocks the plots were arranged entirely at random, and with this limitation the four blocks were replicates of one another.

In an attempt to reduce the observable colour differences to numerical terms, a single block of this experiment was used. A copy of Ridgway's *Colour Standards and Nomenclature* (17) was taken on to the field and a suitable colour scale found by a series of trials. That finally selected was Green- Yellow XVII, 27'. Direct comparison was then made with ten plants on every plot on the selected block. The plants were taken at random and to ensure a fair comparison, the pinnae examined were taken at the same position on the stem and leaf in every case. The selected position was that used for the experiments already described. It was found advisable to make the comparison by diffused light, and to secure this the scale was

shaded from direct sunshine by a hat. From the ten observations an average figure was calculated for each plot, as set out in cross columns below :

TABLE XI.
Average Green Tone.
(NH₄)₂SO₄ in cwt. per acre.

K ₂ SO ₄ in cwt. per acre	0.	1.	2.	4.
0	6.1	6.6	7.1	8.0
1	6.2	7.2	7.1	7.8
2	6.1	6.6	7.1	7.4
4	6.0	6.5	6.7	7.2

The general result is clear without formal analysis. Increased application of ammonium sulphate leads to a deepening of the leaf colour, but the amount of potassium present is without effect. It must be remembered, however, that even though no potassium is added, a certain quantity is present in the soil, and it has in fact been observed (10) that at very low concentrations potassium has an effect on leaf colour. Such observations were obtained with water cultures and are quite impossible under field conditions.

8. SENESCENCE.

The change of colour associated with the senescence of the potato leaf is perfectly definite, and is not affected by such small differences as those of the above section. Although the green colour may fade before its final disappearance, a stage is reached at which a golden yellow appears, and advances a well defined frontier into the green. It is thus easy to judge by direct inspection the proportion of a leaflet that has lost its original pigment. Since the leaf is divided into a number of separate pinnae (usually seven or nine), the proportion of the leaf which has changed is also easy to observe. After yellowing the leaf undergoes a further and final colour change, when it shrivels and becomes a dark brown, and shortly after this it drops from the stem leaving only a scar to indicate its existence. The yellowing and withering of the leaves may be taken as indicators of their metabolic age, and the proportion of yellow and withered leaves on the plant as a similar index to condition. If two plots receiving different treatments, *A* and *B*, are planted on the same day, the plants upon them will not reach maturity at the same time. At any intermediate date, therefore, the *A* plants will be at different stage of their life-cycle from that of the *B* plants. In the case of individuals their relative positions could be assessed during the later phases by determining the amount of yellow leaf surface (*Y*) that had appeared. The shorter lived plant would show the greater amount of colour

change. In dealing with plots and controlled treatments the same method could be applied to a sample of plants taken from each plot at random. The further change to brown could be taken into account in two ways. In the first place browned leaves, including those which had dropped from the plant, could be considered as having fully yellowed and the further changes be ignored in calculating the 'yellow index', $Y + B$. The number of brown leaves (B) could also be taken as an independent measure of age, and the two estimates be compared. Alternatively a brown leaf could be regarded as indicating a greater ageing than a yellow one, and the total age index be obtained by the expression $Y_b = Y + kB$ where k is some constant greater than unity. Both methods were tried, since the same primary data could be used in each case. To estimate Y three categories of leaves were taken into consideration, fully yellow, more than half yellow, and less than half yellow; the values 1, 0.75, and 0.25 being assigned to these respectively. The separation of the leaves into these classes by inspection offered no difficulty, and Y was then obtained for each plant examined by adding together the class values, thus:

$$Y = \begin{array}{ccc} \text{number} & \text{number} & \text{number} \\ \text{fully} & + \text{more than} & + \text{less than} \\ \text{yellow} & \text{half yellow} & \text{half yellow} \end{array} \times 0.75 + \text{less than} \times 0.25.$$

B was obtained by counting the number of withered leaves attached to the plant, and adding the number of leaf-scars which could be detected at the base of the stem. Once withering commenced its progress over the leaf was so rapid that it was difficult to determine categories such as those applied to yellowing, and only whole numbers could be dealt with. The arbitrary value of 2 was assigned to k .

The first series of figures obtained were treated according to both methods of estimating ageing, the results of which were found not to differ in any important respect. The relations between different treatments, that is to say, were identical, whichever method of estimation was used. Counting was carried out on the 'Quantitative Experiment' upon three different occasions, July 23, August 11 and 27. The first two counts were made on block B , and the third on block D . On each occasion the treatments examined were 0, 1, 2, and 4 cwt. K_2SO_4 per acre with no addition of ammonium sulphate. On July 23 ten plants taken at random were examined on each plot, but on each of the subsequent occasions this number was increased to twenty. In each case Y_b was calculated and the estimates analysed statistically. To test the significance of the differences between treatments, each occasion was taken separately, since the number of observations was not always the same. On July 23, 39 degrees of freedom were available from the forty plants examined, three of them being ascribable to treatment and the remaining thirty-six to uncontrolled, or 'random' causes. On both

the following occasions there were 79 degrees of freedom in all, with three still belonging to treatment, and 76 being random. The tests for significance gave the following results:

TABLE XII.

Analysis of Variance of Leaf Ageing.

	Sums of Squares.	Degrees of Freedom.	Variance.	Log _e Variance.	Z Found.	For $P=0.05$.
July 23. Treatment	156.1547	3	52.0516	3.9522	1.0984	0.47-0.55
Remainder	208.3437	36	5.7873	1.7555		
Aug. 11. Treatment	632.2461	3	210.7489	5.3504	1.1839	0.4787
Remainder	1500.5281	76	19.7438	2.9826		
Aug. 27. Treatment	10069.9898	3	3356.6632	8.1189	0.6383	0.4787
Remainder	71177.7344	76	936.5497	6.8422		

On each occasion there is a clear indication that the value of Y_b is dependent upon the quantity of potassium sulphate supplied, and hence that this substance affects the length of the life-cycle of the potato plant. In Fig. 6 are given curves illustrating the mean values of Y_b . It will be seen that on each occasion the middle range shows a reduced amount of yellowing compared with both the high and low extremes. The treatment variance was, therefore, further analysed as follows:

TABLE XIII.

	Sums of Squares.	Degrees of Freedom.	Variance.	Log _e Variance.	Z Found.	For $P=0.05$.
July 23. 0 and 4 with 1 and 2 Other Com- parisons	153.7656	1	153.7656	5.0357	1.6401	0.67-0.72
Aug. 11. 0 and 4 with 1 and 2 Other Com- parisons	2.3891	2	1.1946			
Aug. 27. 0 and 4 with 1 and 2 Other Com- parisons	603.4258	1	603.4258	6.4026	1.7100	0.6729
	28.8203	2	14.4102			
	8226.5820	1	8226.5820	9.0856	1.1217	0.6729
	1843.4078	2	921.7039			

By comparing these variances with the appropriate remainder variances in Table XII, values of Z were obtained as shown. On each occasion the great bulk of the total treatment variance is contributed by the comparison between the middle and extreme values of the range, and only a very small proportion is attributable to other comparisons. As might be expected, therefore, the variance of the selected comparison is shown to be undoubtedly significant, while those of other comparisons are not.

An interpretation of the curves of Fig. 6 seems, therefore, clear. They indicate a case of 'physiological balance', the importance of which has

already been shown in numerous instances. At the medium concentrations potassium is co-ordinated with other substances present in a manner favourable to a prolonged life-cycle, and yellowing and the senescence it indicates are, therefore, postponed. At the higher concentration this balance is again lost, and yellowing is as fast or faster than with a deficiency. A second possible explanation can be disposed of. If the total number of leaves on the plant was also smaller on the 1 and 2 cwt. plots than on the others the form of the curves might simply be due to a uniform yellowing proportional to the total number of leaves produced. In the analysis of leaf number on p. 178, it is shown, however, that there was a significant reduction of leaf number at the top and not at the bottom of the concentration range. These results were obtained at the same time, and using the same plants as the yellowing data. The same result can be shown by calculating Y_b as a percentage of the total leaf number. If the explanation is true, the curve should then become a horizontal straight line, independent of concentration. Actually (see Fig. 6, broken curve) only a relatively unimportant deviation from the former curve is introduced. It thus makes little difference whether Y_b is expressed as an absolute amount or as a percentage of the total number of leaves.

A further aspect of senescence which, on account of its visible results, may be called 'coppering', is brought into sharp relief by these methods. About the beginning of August some of the plants began to develop minute brown spots on their leaves. These were at first extremely small, and scattered irregularly about the laminae, though somewhat more numerous towards the edges. They involved all the internal layers, passing right through the leaf and appearing at a corresponding point on the other surface. With time these spots extended and coalesced into brown, withered patches of irregular form and size, the tissues of which were obviously dead. It was noticeable as they began to enlarge that they were formed, and for a long time restricted, within the islands lying between the veins. Not until a comparatively late stage did they invade bundles large enough to be visible. This method of development is in sharp contrast to the withering that follows the change to yellow. In the latter case the leaf turns brown in much larger patches which work inwards from the border, instead of originating all over the lamina. Coppering, moreover, is not necessarily or even typically associated with yellowing. Whereas the latter starts at the lower leaves and only slowly works its way upwards, coppering appears upon the younger leaves at the top of the haulms, and usually at a stage when they are still quite freshly green. Its occurrence in lower leaves is very much rarer.

A general inspection of the potato plots suggested a close connexion between coppering and a low potassium supply. On August 27, when the change was much in evidence, a count was made simultaneously with those

for yellowing, of the number of affected leaves on each plant. There were thus obtained figures for a sample of twenty plants on each of four plots receiving 0, 1, 2, and 4 cwt. potassium sulphate per acre respectively.

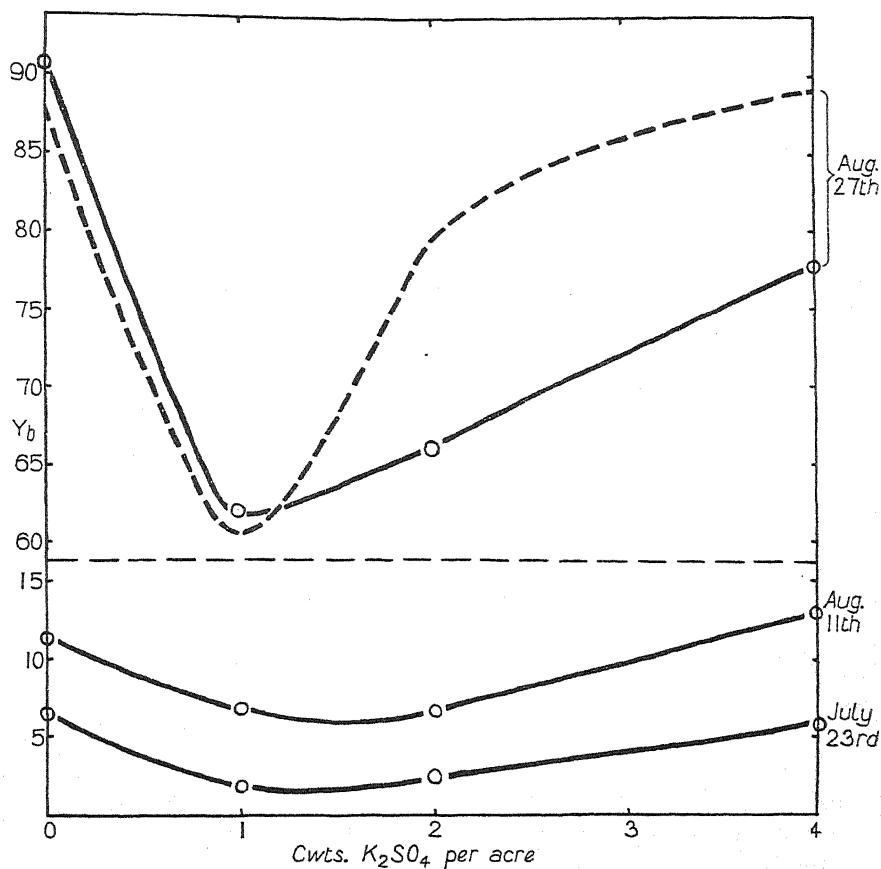


FIG. 6. Age index, Y_b (see p. 190), for three different periods and four different treatments. The curved broken line shows the index expressed as a percentage of the total number of leaves. The scale of ordinates is interrupted between 15 and 60.

Analysis of the variation observed gave the following results:

TABLE XIV.

Analysis of Variance of Coppering.

Treatment	Sums of Squares.	Degrees of Freedom.	Variance.	Log _e Variance.	Z Found.	For $P = 0.05$.
Remainder	1064.8	3	354.93	5.8718	1.3078	0.4787
Totals	1972.0	76	25.95	3.2563		
	3036.8	79				

A significant reduction in the number of affected leaves is thus associated with an increase in the supply of potassium. That the reduction is not dependent to any great extent upon the simultaneous reduction of the total leaf number is shown by its magnitude. The number of leaves on the untreated plot did not exceed those on the 4 cwt. plot by as much as 20 per cent., whereas the number affected by coppering was ten times as great. Another count carried out on August 11 gave similar results with smaller numbers. On the other hand, no such reduction was found when ammonium sulphate was investigated, and there seems every reason to suppose that coppering, or local death of the leaf tissues, is due to an insufficient supply of potassium.

9. DISCUSSION.

The foregoing results, positive and negative, present a picture of some complexity, and one is forced to conclude that the part of potassium in the leaf is not a simple action, but rather a manifold activity. It is now well established that a deficiency of potassium¹ reduces the amount of reserve carbohydrate precipitated by the plant, and the main object of this discussion is to examine the mechanism which brings the reduction about. In the early part of the paper it was shown that adequate potassium manuring causes an increase of efficiency, rather than a multiplication of the photosynthetic machinery. Leaf area is, if anything, reduced by additions of potassium which simultaneously cause larger amounts of starch to be formed in the leaves themselves and in the reserve organs. Laying aside mere increase of leaf surface, attention may, therefore, be concentrated upon the question of efficiency. This may vary in any or all of the stages lying between the uptake of carbon dioxide and the final appearance of starch. For the purpose of the present analysis the following stages may be recognized :

1. The diffusion stage in which carbon dioxide travels to the chloroplast surface. Carbon dioxide concentration is here pre-eminently the controlling variable.
2. The photochemical stage upon which light exerts a controlling influence, and
3. The 'dark reaction' stage, which is subject to the influence of heat.

As a result of these reactions hexose sugars are produced, which may then undergo further modifications, the principal of which is condensation to starch. This may be called the fourth or condensation stage of the series. The first step in the removal of starch from the leaf is assumed to be its reconversion to a hexose sugar, and translocation must not therefore be separated from the question of starch formation.

¹ It is not formally shown by the experimental results that the observed effects are due to potassium and not to the associated sulphate. The evidence of yield experiments is strongly in favour of the kation.

None of these stages is *a priori* ruled out of the problem. In the first, potassium might materially increase the supply of carbon dioxide to the chloroplast by the formation of a bicarbonate buffer in the cytoplasm. It is improbable that such buffers occur, however, since it has been shown that the pH of plant cells is rarely alkaline (Atkins, 1 and 2), and the active intervention of potassium at this stage cannot be substantiated. Evidence of its effect in the photochemical and dark stages is, however, forthcoming from the work of Briggs (5), who found that bean seedlings deprived of potassium showed reduced rates of oxygen production, both when light and when temperature were limiting. Accelerations in these stages, to whatever cause they were due, would lead to an increased production and concentration of hexose, which in its turn would promote a greater formation of polysaccharide. The observation of increased quantities of starch in the leaf does not, therefore, necessarily indicate that the condensation stage itself has been made more efficient, since a faster rate of any step in the series will lead to a quickening of them all. That the efficiency of this stage is directly effected becomes probable, however, from a consideration of starch removal. Both from the work of Maskell (14) and from the present paper, there is reason to suppose that this is also accelerated. The velocities of the hexose-starch and starch-hexose reactions are dependent upon a number of factors, among which may be named temperature, concentration of hexose, and concentration of enzyme. Starch, being only slightly soluble, occurs as a saturated solution in the presence of its solid phase, and its effective concentration is thus kept constant. Fluctuations in the total amount of starch in the leaves are not, therefore, reflected in the velocity of the starch-sugar reaction. Light also is without direct effect, as this stage of the process is able to continue in the dark if sugars are artificially supplied. As temperature may further be ruled out, any effect of potassium upon the rates of the reactions may be expected to take place through the concentrations of hexose or enzyme. If the opposing reactions are supposed to constitute a single reversible reaction, a reduced hexose concentration during a period of darkness might be expected to lead to a faster disappearance of starch. It has been shown by Mason and Maskell (15) that translocation always occurs from high to low concentrations of sugar, and that the rate of translocation is proportional to the fall in concentration. Starch removal might, therefore, be accelerated by a reduction of hexose concentration in the leaves, accompanied by a corresponding reduction in the stems and final storage organs, if any. In the Rothamsted field experiments of 1900 and 1902 (12) it was found that the addition of potassium sulphate led to a larger formation of hexose sugars in the roots of mangolds. Beal and Muncie (4) found that application of the same substance raised the concentration of sugars in sap pressed from the leaves and stems of carnations. The acceleration observed in the removal of starch is,

therefore, not likely to depend on a reduced hexose concentration, and we are driven to explain the additional hydrolysis as due to an increased activity of the appropriate enzyme series, which is diastase.

If, on the other hand, one accepts the evidence which has been brought forward to prove starch condensation and hydrolysis two independent reactions, hexose concentration can have no direct effect upon the rate of starch decomposition, and again, the action of potassium can only be through the hydrolytic enzyme. This explanation is made even more probable by the demonstration that diastase *in vitro*, when completely separated from neutral salts becomes inactive, and that its activity is restored by addition of salts of potassium and other metals (Vulquin and Lisbonne, 19). The results of Kendall and Sherman (13) and others make it highly probable that diastase accelerates the condensing reaction as well as the hydrolysis, and the presence of potassium will therefore influence them both.

There is thus reason to believe that potassium directly affects three of the four stages in starch formation, and it would be interesting to know whether this is done by similar or widely different means. In the last stage, it has been suggested, there is an increased effectiveness of catalytic surface, but the manner in which this is secured is not clear. The alternative possibilities appear to be four. There may be an actual increase of the enzyme surface owing to (1) a greater dispersion of its material, or (2) the formation of entirely new enzyme; or there may be an increased efficiency of a unit area of the surface already existing, (3) by an increase of its adsorbing or combining power, or (4) by a change in the degree of dissociation.

In his examination of the photochemical and dark stages of assimilation, Briggs came to the conclusion that the effect of potassium and other metals was to increase the active chloroplast surface, and a certain amount of unity can thus be recognized in the potassium effects in the various stages of carbohydrate metabolism, since they are always concerned with an increase of catalytic powers.

None of the facts elucidated in the foregoing paper or otherwise known to the author is in any way at variance with this conclusion. It is not supposed, however, that this represents the sum of the activities of potassium ions in the plant, and the effect upon the rate of yellowing is a case in point. The ageing of leaves has been found to be correlated with the disappearance of such diverse substances as water (8), nitrogen (6), carbohydrates, chlorophyll, and many mineral elements of which potassium (7) is one. Most of these pass into the axes of the plant, others such as chlorophyll are degraded *in situ*. In most instances the reason for migration or decomposition is quite unknown, and cause cannot yet be distinguished from effect. Lack of carbohydrate is presumably a result of leaf degeneration, not a primary cause of it. With the ash elements the case is not so clear, and it is there-

fore interesting to find, as shown above, that an increased supply of potassium will delay the breakdown of the mechanism. Here, it seems, is a factor which can definitely be said to be controlling, not controlled.

To describe the way in which this is done is another matter. In a process governed by so many factors, known and unknown, the attempt, without further knowledge, can only lead to disappointment.

✓✓ 10. SUMMARY.

1. In order to investigate the physiological importance of potassium, field experiments were carried out upon certain attributes and functions of potato leaves. Number, area, weight, water content, and rates of starch formation, translocation, and senescence were examined. The primary data were subjected to statistical analysis, and the following conclusions arrived at.

2. The number of leaves formed on an average per plant was found to be significantly reduced by the application of potassium sulphate, or 'potash manure salts', a low-grade fertilizer. Potassium chloride could not be shown to have any effect.

3. Area of a selected leaflet. The area of the penultimate pinnae of the fourth leaf from the stem apex was not affected in adult plants by the addition of potassium sulphate, but addition of 'potash manure salts' or potassium chloride caused an increase of surface. This is ascribed to the action of the chloride ion present in both the latter fertilizers. Taken in conjunction with the reduced leaf number, the lack of effect of the sulphate suggests that potassium itself tends to decrease rather than increase the total leaf area of the plant. There is, however, some evidence of an increase in the earliest stages of growth.

4. Leaf water content, expressed as water weight / dry weight, showed no significant response to potassium manuring. The presence of chlorides, however, again caused an increase. It is shown that a very high correlation exists between leaf area and the water weight / dry weight ratio, and the increase of leaf area due to chlorides is probably brought about by an increase of water content.

5. Dry weight of the selected leaflet was found to be unaffected by the addition of potassium compounds.

6. Starch formation per unit leaf area showed a significant increase in response to potassium, particularly when in the form of sulphate. There was little or no response to the presence of chlorine.

7. Translocation could not definitely be shown to be affected by the same treatment, but reasons are given which make it probable that an acceleration in its rate is brought about

8. Senescence, as indicated by the yellowing of the leaves, was delayed

by the addition of one or two cwt. of potassium sulphate per acre. Four cwt. per acre did not have a similar effect. In all these concentrations there was no detectable effect on the colour of healthy green leaves. 'Coppering', a characteristic spotting of young foliage, was shown to be clearly related to a deficiency of potassium.

9. These points are discussed, and it is shown that one important effect of potassium in leaves is an increase of catalytic activity, leading to greater efficiency in three of the four stages of starch formation. It is further suggested that loss of potassium is a causal factor in leaf ageing.

The author wishes to express his great indebtedness to Dr. R. A. Fisher for his invaluable advice on the statistical handling of the data, and to the many willing helpers who made their collection possible.

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Nuclear Division in the Plasmodiophorales.

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With Plates XV and XVI and one Figure in the Text.

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I. INTRODUCTION.

IN 1899, Nawaschin (41) described with precision his observations on the life-history and cytology of *Plasmodiophora brassicae* and various nuclear configurations associated with the development of the organism from the myxamoeba to the spore-producing coenocyte.

Since 1899, similar nuclear configurations have been observed and misinterpreted by Prowazek (49, 50), and others (38, 6, 21, 47, 68, 10). Certain of these writers have, in addition, and on very meagre evidence, postulated both nuclear fusion and reduction in the genera studied. These later contributions, taken collectively, register no very marked advance in a knowledge of nuclear structure. Hindered by the small size of the nucleus and the difficulties presented by the technique essential to the study of microscopically minute structures, the various authors by imperfect observation and indiscreet interpretation have, in the opinion of the writer, obscured and confused the issue. It is, indeed, difficult to obtain a clear conception

of the actual sequence of nuclear events in any one of the genera they have examined or to attempt a comparison of the various genera with any degree of accuracy.

The foregoing criticisms will be supported in the course of this paper by evidence obtained by the present writer some years ago when investigating the cytology of *Spongospora*, *Sorosphaera*, and *Plasmodiophora*. Some of the results¹ were announced at Portsmouth in 1911 (29): it was shown that the nuclei in the soma divide by karyokinesis and that four V-shaped chromosomes are concerned in the mitosis. It was discovered subsequently that the synaptic effect which had led previous writers to *assume* that meiosis occurs in the Plasmodiophorales is partly due to a delayed union of opposite sets of four V-shaped haploid chromosomes and that the diploid state is immediately followed by meiosis. The reconstruction of the life-history presented in the following pages depends primarily on the recognition of the number and shape of the chromosomes, and for this reason the nature of the nuclear changes and general sequence of nuclear events in the Plasmodiophorales should be established beyond reasonable doubt.

II. MATERIALS AND METHODS.

The *Spongospora* material was obtained from potatoes grown at Cleadon Grange, Durham, in September and October, 1910. *Sorosphaera* was found in galls on *Veronica Chamaedrys*, in Devonshire and Kent. *Plasmodiophora* was obtained from various sources at different times.

The material principally used was fixed a few hours after collection in Flemming's weak solution. Various stains were employed, but finally reliance was placed on Flemming's triple stain and on Heidenhain and Orange G.

The critical nuclear configurations were elucidated with the aid of Zeiss apparatus including the 2 mm. (N.A. 1.40)² and 3 mm. (N.A. 1.40) objectives and compensating oculars, 2 and 18. Various sources of illumination and suitable colour screens were employed. The 2 mm. or 3 mm. objectives combined with compensating oculars 6 or 8 proved most useful for studying the general detail. For special three dimensional detail, compensating ocular $\times 18$ was employed, using the draw-tube either closed or fully extended. Outline drawings of the various nuclei and nuclear objects were usually made at this high magnification with the aid of an Abbé camera lucida. The figures of dividing nuclei, &c., found in the plates, were reconstructed from diagrams and drawings made at various magnifications, after repeated verification. With the object of confirming some of the conclusions reached a model spindle with adjustable 'chromosomes' was constructed.

¹ The delay in publishing the full results was unavoidable and not due in any way to the author's lack of confidence in them.

² The author is greatly indebted to the Royal Society for the loan of a Zeiss 2 mm. (N.A. 1.40) objective.

III. THE SOMATIC PHASE.

(a) *The Development of the Soma.*

The sequence of the developmental changes undergone by any organism during its life-cycle can be established with certainty only by direct observation of the succession of growth forms in time. With endocytic parasites the course of events in time may be ascertained by employing some such technique as that used by Curtis (*Synchytrium*) who observed the growth forms within the host cells at varying time intervals from infection. The work on the Plasmodiophorales is, however, based on a less secure foundation, since knowledge of the sequence of development has been derived from a comparison of series of forms obtained at any one time and then arranging these in supposed chronological series. In this study it has been assumed that the stages in the organism are correlated with increasing size of the parasitic growth occasioned, and hence by arranging the growths in order of increasing size, successive stages of the life-history were obtained. In part, the reconstruction of the events in the life-history of the parasite was guided by the stage of senescence shown by the cells in which the individuals were found. It is thus seen that the reconstructed order is not arbitrarily dictated by preconceived notions on the author's part, but is based on objective evidence obtained from phenomena directly associated with the presence of the organism studied and capable of arrangement in a time sequence.

In the case of *Spongospora*, only uninucleate myxamoebae were observed in sections taken from the smallest wart-like excrescences ((30), Fig. 99 a) on potatoes affected with the parasite. Such minute galls result from the local numerical increase of the host-cells, a point established by observing that in any given section, several of the host-nuclei were in a state of division. The dividing cells almost invariably contained myxamoebae and, in late stages, myxamoebae were observed on both sides of the divisional membrane. Hence the host-cell contributes a quota of its endocytes to each of its daughter nuclei. This method of distributing the parasite is sufficient to explain the even distribution of infected cells throughout the gall.

With regard to the multiplication of myxamoebae within the host-cells, the possibility of mass infection must be borne in mind. Indeed, from the fact that many potatoes showed large superficial areas of infection ((30), Fig. 101 c and d), mass infection seems highly probable. In nearly all the sections examined numerous cells were infected, and the myxamoeban numbers for different cells varied greatly. The evidence for actual multiplication is provided by the observed fact that the nuclei of the myxamoebae divide, and that the division is followed by the segmentation of the cytoplasm, each of the two segments thus formed containing a

daughter nucleus. It is obviously impossible to gather any idea of the number of generations of myxamoebae formed, and no indication is given by the myxamoeban numbers. Olive ((46), p. 463) describing the living myxamoebae of the Acrasieae states: 'Just how many times division of a single individual and of its derivatives may occur can hardly be determined without continuous observation through several days; the number undoubtedly varies, however, with the amount of available moisture and food.'

When somewhat larger and older galls ((30), Fig. 100c) are examined, it is seen that plurinucleate as well as uninucleate amoebae are present in the majority of the host-cells. These coenocytic amoebae show no signs of cytoplasmic segmentation. Thus a change has taken place in the nature of the growth-forms produced in time, which seems to indicate a lessening of developmental activity within the host-cells where the coenocytes are found.

In the case of *Sorosphaera*, Maire and Tison (38) state that the earliest growth-forms observed are those of myxamoebae and that each cell is infected with one or more of these amoebae. The myxamoebae become at first quadrinucleate and then eight-nucleate and occasionally as many as, but not more than, sixteen dividing nuclei were observed: the number of nuclei, however, is not always a multiple of four. These coenocytic amoebae are styled schizonts. The schizonts by fragmentation, which is frequently very irregular, give rise to uni- or plurinucleate meronts: they commence to fragment when they have reached the eight-nucleate stage. The uninucleate meront is equivalent to the original parent amoeba and pursues the same course of development, while the plurinucleate meronts are able to fragment again after nuclear multiplication has taken place. It is necessary to point out, however, that Maire and Tison's interpretation of the growth changes in *Sorosphaera* is not substantially supported by direct evidence, e.g. evidence showing that the postulated segmentation of the cytoplasm does actually occur, and further that it is undoubtedly influenced by the general resemblance of the growth-forms in *Sorosphaera* to those recorded for the Sporozoa. Winge (68), referring to both *Sorosphaera* and *Sorodiscus*, was quite unable to state with certainty whether the uninucleate myxamoebae under observation in his preparations were really primary amoebae or meronts derived from the division of schizonts, and the question as to whether or not there was an increase in myxamoeban numbers during the period immediately following the infection of the schizonts was left unanswered. The present writer's observations on the amoebae of *Sorosphaera* have left him equally uncertain, since the coenocytic amoebae might be either of the schizont type or of a kind which does not subsequently segment to form uninucleate myxamoebae, as in *Spongospora*. In his opinion it would be extremely difficult to establish the cyclic events described by Maire and Tison on the evidence derived from a comparison

of growth-forms present at any one time. The difference in these early developmental changes as recorded for *Spongospora* and *Sorosphaera* respectively is in any case not very great; it is due to this: in *Spongospora* each parent myxamoeba divides to form two daughter uninucleate myxamoebae; in *Sorosphaera*, on the contrary, the division of the myxamoeba does not immediately follow mitosis, the myxamoeba becomes coenocytic and presumably the segmentation of the cytoplasm is delayed for a time.

With regard to *Plasmodiophora* it is also uncertain whether an increase in myxamoeban numbers follows infection. The earliest developmental stages as observed by the writer are very similar to those illustrated in Nawaschin's paper ((41), Figs. 1-5), viz., stages in development of coenocytic amoebae.

Considerably older *Spongospora* galls ((30), Fig. 102, *e-f*) show a preponderance of relatively large coenocytes in the host-cells. These are analogous to the plasmodia of *Plasmodiophora*, but it is extremely difficult to establish by direct observation that the larger growth-forms have been formed by the fusion of smaller ones. They may, alternatively, have developed from smaller ones or they may be merely coenocytes in close approximation. Nevertheless, the author holds the view, for reasons which will be given later, that a fusion of coenocytes in certain circumstances does actually take place.

(b) *The Somatic Mitoses.*

The somatic nucleus in *Spongospora* is approximately spherical and somewhat variable in size. It is bounded by a distinct membrane. When viewed in any median plane it presents a configuration resembling a wheel with the more or less centrally situated nucleolus corresponding to the axle and portions of nucleoplasm or nuclear substance representing the spokes (Pl. XV, Fig. 1). From a three-dimensional point of view the nucleus shows a chromatic, spherical nucleolus from which oblique, curved, achromatic films of nucleoplasm (linin) extend to the nuclear membrane. The surface of the nucleolus is not entirely smooth; here and there minute, cylindrical, chromatic projections are evident which appear to follow the alinement of the linin films. Similar curved chromatic rods have also been observed between the nucleolus and the nuclear membrane, in close juxtaposition to the membrane, and in the cytoplasm. The shape and curvature of these chromatic rods can only be made out with the aid of the highest magnifications and very careful use of the fine adjustment. The 'chromatin granules' of the somatic nucleus are merely the ends or portions of the chromatic rods presented to the observer when the interior of the nucleus is viewed in any given plane.

The somatic nucleus in the three genera, *Spongospora*, *Sorosphaera* (Pl. XV, Fig. 2) and *Plasmodiophora*, is of the same 'wheel' type, and in this

respect resembles the nucleus in certain Amoebae and in the 'miniature yeasts' found in the human intestine (Pl. XV, Fig. 6). It is, however, quite unlike the nucleus in certain Entamoebae, notably *E. histolytica* and *E. coli*. With these species the nuclei show an alveolar structure and the nucleolus¹ is relatively small (Pl. XV, Figs. 3-5). It is interesting to note from the work of Curtis (11) that the nucleus, in a single species (*Synchytrium endobioticum*) may present alternately an alveolar and a wheel-like form during organic development. Thus the alveolar type is present in the youngest prosorus ((11), Pl. 12, Figs. 10 a-10 d); the latter type during the process of nucleolar discharge which precedes the migration of the protoplasm ((11), Pl. 12, Figs. 14, 15, &c.).

Any section taken through a gall where *Spongospora* is present only in the form of myxamoebae, shows that the great majority of the nuclei² are not in the mitotic state. It may be inferred, therefore, that the time intervals between successive mitoses are much longer than the period of duration of the division itself. The nuclei as observed during the inter-mitotic periods exhibit individual differences with regard to the disposition of the chromatic rods. Since all the nuclei were fixed at the same time these structural differences undoubtedly represent stages in a continuous process of structural change³ of the nucleolus. The nucleus during the inter-mitotic period is therefore in no sense regarded by the writer as a static nucleus, the state implied by Cook's use of the expression 'resting nucleus' in his account of *Ligniera* (10).

The somatic divisions in the higher plants have been worked out by fixing active tissues from time to time as growth proceeds. Moreover, modern methods of technique have permitted observation of the sequence of changes which takes place during nuclear division in *living* cells. Hence the events which in general characterize mitosis, and the order in which they happen, is well established. In the case of organisms such as those dealt with here (and the remark applies with equal force to the great majority of the fungi) the nuclei are exceedingly small, and, as previously mentioned, actively dividing nuclei are of infrequent occurrence. Hence certain stages in division are inevitably rarely found, while others may not be encountered at all. For these reasons it is not always easy to ascertain, from direct

¹ Thus in the case of *E. histolytica* (amoeba) a nucleus 7μ in diameter showed a nucleolus, by volume only approximately one-eighth that of the average and smaller nucleus of *Spongospora*.

² Cook (*Ligniera*) estimates that only one in every five thousand amoebae were undergoing nuclear division.

³ See Digby (12), in connexion with the translocation of nuclear material from the nucleolus to the cytoplasm in *Galtonia candicans*. Curtis (11) found that the nuclei in the young prosorus of *Synchytrium* passed through alternate periods of nucleolar extrusion with subsequent solution of chromatic material in the nuclear sap, and accumulation of chromatic material in the nucleolus, normally, prior to the migration of the prosorus and prior to nuclear division. These nuclear changes were influenced to a certain extent by environmental conditions.

evidence, the order in which the observed events follow each other during division. The author has therefore felt it advisable to rely for guidance in interpreting the mitoses in *Spongospora* on established records, and to present the various divisional configurations in the order suggested as a result of studying the details of somatic mitoses in the higher plants provided by different authors. For convenience somatic mitosis in *Galtonia* has been accepted as a typical case for the higher plants.

According to Digby ((18) p. 733): 'The general object and character of the prophase is to compass a gradual and ever-increasing concentration of the linin until the completion of the fully formed chromosomes. . . . This early concentration may take diverse forms, the linin may be condensed into bands, or into irregular masses, or into granules. . . . In *Galtonia* the chromatin . . . is equally diffused throughout the linin. . . . Concentration proceeds, and the parallel portions of linin, whether they be rows of granules or paired threads, gradually condense to form more homogeneous lengths of spireme. . . . As concentration proceeds, a curling spireme results, the segments of which only straighten out during their final thickening prior to the evolution of the chromosomes.' The few configurations which have been observed indicate that an analogous concentration of nuclear material occurs during the prophase in *Spongospora*. The earliest stage of the prophase is marked by the thickening of the chromatic rods projecting from the nucleolus. In some configurations these are short (Pl. XV, Figs 7, 10); in others, they reach the nuclear membrane (Pl. XV, Figs. 8, 9). Certain configurations very seldom observed show an irregular chromatic network (Pl. XV, Figs. 11, 12) which is the only intermediate stage obtained between the earliest prophase and the spireme (Pl. XV, Figs. 13, 14).

To return to *Galtonia*; during the process of condensation the linin fragments gradually retreat from the nuclear periphery and spread themselves across the nuclear cavity. 'Gradually the individual fragments unite end to end until the typical and definite sixteen chromosomes can be identified.' At the time when the chromosomes go on to the spindle 'the nucleolus is drawn up on the spindle in the confusion of the chromosome movement. . . . When the chromosomes have arranged themselves on the equatorial plate, the nucleolus is pushed off the spindle. . . . It must finally fragment with amazing rapidity . . . but as they (the chromosomes) complete their equatorial arrangement, and as they separate and proceed to the poles, the nucleolus vanishes, and leaves no apparent trace of its existence. . . . The chromosomes attach themselves to the spindle by one end *and for a short space of time they may lie horizontally, that is to say at right angles to the plane of the spindle*' (The italics are the present author's.) The stages in the elaboration of the chromosomes from the spireme have not been observed in *Spongospora*. Several configurations show the chromosomes after they have emerged from the spireme (Pl. XV, Figs. 15-18) and several

have been observed which show at least three V-shaped chromosomes disposed equatorially and prior to the appearance of the spindle fibres (Pl. XV, Figs. 19, 21, and 23). Other configurations show only two objects resembling chromosomes (Pl. XV, Fig. 25). Many configurations resembling those shown in Figs. 19–23 presented at first sight an appearance of a nucleolus with either one or two ‘bars’ of chromatin situated laterally: in some cases a single ‘bar’ appeared to be either in front of, or behind, the nucleolus; and in others, two ‘bars’, one to the right and the other to the left of the nucleolus.

It was eventually found that these chromatin ‘bars’ were not always continuous, the effect of continuity was produced by the close approximation of two or more chromosomes end-to-end. The following illustrative examples are given below:

Pl. XV, Fig. 20. One ‘bar’—three chromosomes, of which that on the right-hand side is inverted showing the two ends.

Pl. XV, Fig. 22. Two ‘bars’—the bar to the right consists of two chromosomes, that to the left shows only one distinct chromosome and a fragment of the second.

Pl. XV, Fig. 23. Two ‘bars’—that to the right consists of two distinct chromosomes; that to the left, of one or perhaps two joined end-to-end.

Pl. XV, Fig. 25. Two ‘bars’—each ‘bar’ is regarded as bivalent.

Pl. XV, Fig. 26. One ‘bar’—probably three or four chromosomes joined end-to-end.

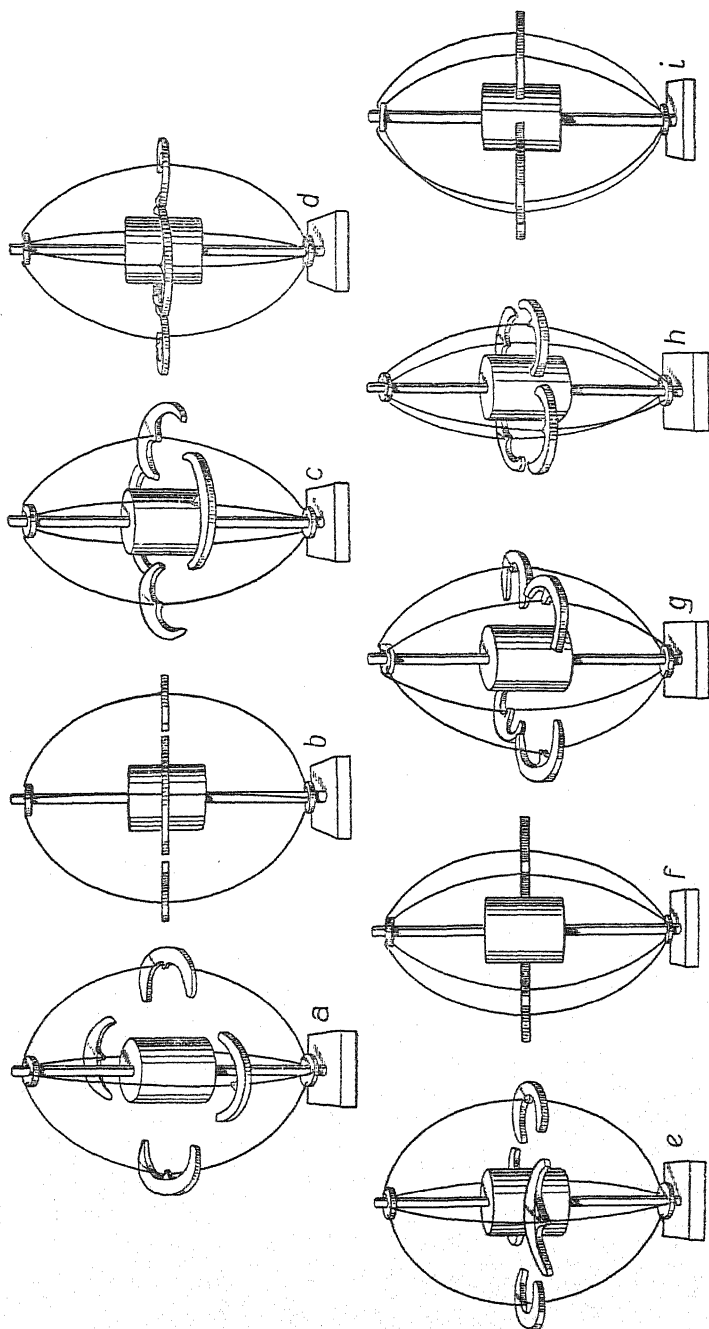
Using the model with four adjustable ‘chromosomes’ to which reference has already been made, the ‘chromosomes’ were arranged in various positions at the equator of the model, viz., in pairs in contact at the ends, or three placed end-to-end and the fourth behind the model nucleolus, and so on. In each case the model was photographed, viewed either from above, from the side, or obliquely, with the result that the optical appearances presented by the various divisional configurations described above were faithfully imitated. Diagrams made from some of these photographic records are given in the Text-fig. *a-i*. The further details are as follows:

a. The model chromosomes are not in contact. The model is viewed obliquely and all the chromosomes are visible.

b. An equatorial view of Text-fig. *a*. The effect produced is that of an almost continuous bar situated in front of the nucleolus.

c. One chromosome in front and three in contact behind the nucleolus. The model is viewed obliquely.

d. An equatorial view of Text-fig. *c* taken from behind the model. The bar is continuous, but the effect produced closely resembles that shown in Text-fig. *b*. See Pl. XV, Fig. 26.



TEXT-FIGURE.

e. The chromosomes are not in contact, the one in front is inversely orientated. The figure is a little oblique. When in equatorial view the one-bar effect is produced; see Pl. XV, Fig. 20.

f. All the chromosomes are in contact, producing the one-bar effect. The bar will appear in front of, or behind, the nucleolus, according to the position in which the model is placed; see Pl. XV, Fig. 26.

g. Two chromosomes in contact on the right, and two in contact on the left of the nucleolus. The model is viewed obliquely.

h. Text-fig. *g* viewed from the side.

i. The grouping shown in Text-figs. *g* and *h* in equatorial view giving the two-bar effect; see Pl. XV, Figs. 22, 23, 25, &c.

One of the configurations most frequently encountered is the peculiar cross-shaped figure or 'Saturn stage' (Pl. XV, Figs. 27–32). The 'Saturn' appearance is due to the union of four chromosomes end-to-end to form an equatorial band which is situated immediately within the nuclear membrane and encircles the nucleolus. In these circumstances, therefore, the ratio of the sum of the lengths of the chromosomes (nL) to the circumference (C) of the spindle at its equator, is unity, i.e.¹ $nL/C = 1$. Since C in this case almost corresponds to the circumference of the nucleus, the approximate length of the individual chromosome can be ascertained: thus for a nucleus 5μ in diameter, the value of L is 3.93μ , and so on.

The actual spindle is extremely difficult to distinguish, firstly because the chromosomes rest against the nuclear membrane and the spindle fibres appear to follow the alinement of the membrane; and secondly because the linin fibres show up as fine lines (Pl. XV, Figs. 23, 29, 30, &c.) and are liable to be mistaken for spindle fibres. Spindle fibres have been recognized in unipolar spindle configurations (Pl. XV, Figs. 24, 27)—the 'balance-scale' figures of Nawaschin (41)—and in a very few others (Pl. XV, Figs. 20, 22, and 30).

Throughout the metaphase and anaphase, the nucleolus is distinct and stains strongly with iron haematoxylin, but not as intensely as do the chromosomes. The nucleolus is either more or less spherical (Pl. XV, Fig. 28) dumb-bell shaped, (Pl. XV, Fig. 32) or cylindrical (Pl. XV, Fig. 31). The variable forms assumed by the nucleolus led Nawaschin to state that it divided into two spherical bodies (during the metaphase) and that these bodies subsequently reunited (during the anaphase) to form a short rod-like nucleolus.

During the anaphase, the chromosome band divides to form two daughter bands which move apart from one another in the direction of the poles of the spindle. The daughter bands become discontinuous during the journey and each eventually breaks up into four chromosomes. Several

¹ For convenience this ratio will be referred to as R .

configurations of the nucleus in anaphase were observed, the more important of which are considered in detail below :

- Pl. XV, Fig. 33. Six daughter chromosomes are evident, the remaining two are probably masked by the nucleolus.
- Pl. XV, Fig. 34. Two segments were observed in the upper and three in the lower portion of the nucleus.
- Pl. XV, Fig. 36. The daughter bands are almost continuous.
- Pl. XV, Fig. 37. The upper band is continuous; the lower is discontinuous and two segments are evident.
- Pl. XV, Fig. 38. Four chromosomes are evident in the upper portion of the figure, the fourth chromosome in the lower portion is probably masked by the nucleolus.
- Pl. XV, Fig. 39. Three segments are present in the upper, and two in the lower portion of the figure.
- Pl. XV, Fig. 40. Six daughter chromosomes are evident, two are either masked by the nucleolus or absent from the section.
- Pl. XV, Fig. 41. The only configuration in which the two arms of the V have been observed in the same horizontal plane. The median and lateral upper chromosomes are exceedingly distinct. The fourth upper chromosome was probably absent from the section. The presence of the three upper chromosomes in this configuration was confirmed by both Sir John Farmer and Miss Digby, in 1911.
- Pl. XV, Figs. 42-4. Two chromosome segments are present in the upper part and two in the lower part of each figure.

Numerous configurations of the nucleus in telophase were observed, and in many cases the daughter chromosomes could be distinguished (Pl. XV, Figs. 45-9). During the telophase the nucleolus divides to form two daughter nucleoli (Pl. XV, Figs. 45-9).

It is perhaps noteworthy that nuclei containing a spherical nucleolus are usually those in which separate daughter chromosomes may be observed in early anaphase, whereas in the case of nuclei showing a cylindrical nucleolus the daughter chromosome bands are either continuous or indistinctly interrupted. In the latter case each band often breaks into two segments towards the end of the anaphase, and the segments presumably again break to form the four daughter chromosomes. The nuclei, therefore, appear to be of two slightly different types. It should be remembered, however, that the description of the somatic nuclear divisions given above is a composite one, compiled as it is from the observations made on more than one mitosis in time. The variation in the observed form of the nucleolus as well as the variable disposition of the chromosomes recorded during the metaphase and anaphase may simply be related to the time

factor, and the condition of the nuclei wherein the appearance of the daughter chromosomes as separate individuals is delayed may be an age effect.

As far as *Sorosphaera* is concerned, dividing nuclei were found to be of very rare occurrence in the material examined, and although the staining methods employed were similar to those adopted for the investigation of the somatic mitoses in *Spongospora*, the detailed structure could not be easily made out in the few divisional configurations observed owing to the darkening of the membrane by the stain. The further removal of stain resulted in the decoloration of the chromatic constituents of the nuclei, and consequently it was quite impossible to study the somatic mitoses in detail. With regard to *Plasmodiophora*, the various nuclear configurations described by Nawaschin were observed in sections through very young turnip galls prepared in 1909. These sections passed from the writer's possession and were not subsequently recovered. *Plasmodiophora* was again studied in 1912, but the parasite had unfortunately reached a late stage of development in the galls from which the sections were taken, and only post transitional mitoses¹ were encountered. The nuclei in *Plasmodiophora* are smaller than those of *Spongospora* and less suitable for the purpose of a detailed study of nuclear division.

IV. TRANSITIONAL PHASE.

After an undetermined period of mitotic activity, the somatic nuclei in *Spongospora* cease to divide, and the various growth forms present in the cell undergo a curious change. It is seen that the nucleolus, hitherto the most prominent feature of the nucleus, gradually loses its affinity for the iron haematoxylin stain until it is no longer easily recognized. Concurrently, the cytoplasm becomes increasingly chromatic, an effect due to the presence of numerous stainable granules (chromidia) which are larger than those usually observed during the somatic phase (Pl. XV, Figs. 50-3; Pl. XVI, Fig. 54). During the time when these alterations in the nucleolus and the cytoplasm are taking place, chromatic projections from the nucleolus along the linin films and 'chromatin granules' associated with these films may be observed, differing from those evident during the intermitotic periods (somatic phase) merely in bulk.

V. THE MEIOTIC PHASE.

The post-translational or meiotic nucleus (Pl. XVI, Fig. 55), the 'Geschlechtskern' of Prowazek, the 'new nucleus' of Osborn, the 'reconstructed'

¹ Chromosomes were observed in metaphase configurations. It was not possible to ascertain their exact number. There are, however, not less than four chromosomes.

nucleus of Cook, is of the usual 'wheel' type, but the nucleolus so prominently developed in the somatic nucleus is relatively inconspicuous. Well-marked asters and centrosomes are present at opposite ends of the nucleus. Asters and centrosomes are absent from the somatic nucleus of *Spongospora*, but they are shown by the somatic nucleus of *Sorosphaera* (Pl. XV, Fig. 2). The presence of centrosomes and asters is therefore not necessarily a distinctive character of the meiotic nucleus.

The first indications that the nucleus (*Spongospora*) is undergoing change are evident in configurations which show the presence of groups of curved, ill-defined, chromatic, rod-shaped bodies in the vicinity of the centrosomes (Pl. XVI, Figs. 56-8). Such figures are connected with others (Pl. XVI, Figs. 59-64) which show a more or less looped arrangement of the chromatin, and the loops appear to be linked together by achromatic threads (linin threads). All these configurations exhibit distinct bipolarity in the disposition of the chromatic substances, and a relatively inconspicuous nucleolus is present. Bipolar groups of four distinct V-shaped chromosomes were present in certain obviously later configurations (Pl. XVI, Fig. 65). Such figures were linked by intermediate ones (Pl. XVI, Figs. 66-8) to others (Pl. XVI, Fig. 69) which show eight chromosomes grouped together at one side of the nucleus. The nucleolus was absent throughout these later stages (Pl. XVI, Fig. 65, onwards). These configurations are evidently of a diploid nature, and the grouped arrangement which resembles a contraction figure is undoubtedly due to the approximation of two sets of four haploid chromosomes. Similar configurations were probably observed by Maire and Tison (38), in *Sorosphaera*, and by Winge in *Sorodiscus* ((68) bipolar arrangement of the chromatin in 'garlands'), but these authors appear not to have appreciated the peculiar significance of the varied dispositions of chromatin shown in their illustrations.

The writer has been unable to follow the nuclear changes which take place between the stages represented in Pl. XVI, Fig. 69, and diakinesis. Numerous configurations have been observed in which the chromatic materials are massed together, and present appearances suggesting synapsis. In the majority of cases the appearances proved to be quite deceptive, being effects presented by nuclei showing various stages of change in different circumstances: for example, when the nuclear axis is inclined, when certain nuclei are more or less superimposed or approximated, &c.

The failure to obtain the stages leading from synapsis to diakinesis is perhaps not surprising in view of the rarity of occurrence of certain stages in the somatic mitosis. The details of the somatic division were studied in sections of host tissue taken from very young galls where, as previously mentioned, the parasite was present in the form of myxamoebae or coenocytic amoebae, and consequently the mitoses observed in a given section were all of the same kind (somatic). Nevertheless, certain stages in the

division were seldom observed, for example, the spireme stage which was observed only three times, although many thousands of nuclei were examined. The chance of obtaining a rare or ephemeral stage in the first meiotic division proved to be even more remote, because the sections examined were taken from older galls where all the later developmental stages in the life-cycle were present, viz., those ranging from the akaryote stage to spore formation. Hence only a relatively small proportion of the growth forms in the sections showed stages in the first meiotic division.

The stage of diakinesis (*Spongospora*) has been only once encountered, but in this single instance the nucleus was quite clearly outlined, and the disposition and shape of the chromosomes seemed quite distinctive. As shown in Pl. XVI, Fig. 70, the chromosomes are set apart from one another, they are relatively short and thick, and vary in shape. One of the chromosomes is definitely ring-shaped. Fig. 70 should be compared with those representing diakinesis in *Osmunda* and *Aneura* (Farmer and Moore (18), Pl. XXXV, Figs. 26, 27; Pl. XXXVI, Fig. 35), in *Galtonia* (Digby (13), Pl. LXII, Fig. 64), and in *Gnomonia erythrostoma* (Brooks (7), Pl. XLIX, Fig. 30). Ring-shaped chromosomes are recorded by Farmer and Moore in the cases cited above, by Wilson (67)—in *Mnium hornum*, Lloyd Williams (66) in the Dictyotaceae, and by numerous other writers.

The spindle configurations (metaphase) observed in the plasmodia are of three types, of which the second and third are clearly related. These types show distinctive characters which admit of their consideration in chronological order without any risk of error. The earliest configurations (Pl. XVI, Figs. 71-4) show distinct spindle fibres and centrosomes, and the four equatorially arranged chromosomes are well defined, partly owing to the fact that the nucleolus is absent. The chromosomes are short and thick, and there are often very definite gaps between them. In the few cases where it was possible to study individual chromosomes in detail, the chromosomes were usually U-shaped, as shown by Farmer and Shove in *Tradescantia* during the heterotype division ((19), Pl. XLIII, Fig. 45). The chromosomes shown in Pl. XVI, Fig. 74 are of particular interest in that the right- and left-hand chromosomes follow the alinement of the corresponding spindle fibres. The orientation resembles that found in *Osmunda* (Farmer and Moore (18), Pl. XXXV, Fig. 28). According to Farmer and Moore the effect is due to the fact that the daughter chromosomes (heterotype) cling together by one end equatorially, and it marks the commencement of the diaster. Brooks in *Gnomonia* ((7), Pl. XLIX, Fig. 33), P. W. Carter, in *Padina Pavonia* ((9), Pl. VIII, Fig. 16), and Fraser in *Humaria rutilans* ((24), Pl. XXII, Fig. 30) observed similar effects.

The metaphase is followed by some very striking bipolar configurations

¹ It was not always possible to observe four chromosomes. The fourth chromosome may have been left out of the section or perhaps lost during manipulation.

(Pl. XVI, Figs. 75-81). These are very readily distinguished from the bipolar configurations that precede synapsis. Even when casually examined the chromosomes show up as dark granules in the neighbourhood of the poles, and are more prominent than the pre-synaptic, haploid chromosomes. When examined with great care, it is found, on varying the focus, that each individual chromosome shows usually four blunt ends. The significance of this discovery will be apparent from the following passage in Farmer and Moore's paper ((18), p. 514: see also Pl. XXXV, Figs. 16, 17) relating to the diaster: 'The chromosomes in question assume the form of V, but each is seen to be completely split throughout its entire length. Such a figure is produced when a heterotype chromosome becomes attached by its middle instead of by the end to the spindle fibres. Although such figures are rare in the lily, they are quite common in *Tradescantia*, and also in the salamander, as was long ago figured and described by Flemming.' There is thus little doubt that the configurations illustrated in Pl. XVI, Figs. 75-81, represent stages ranging from the late anaphase to the telophase in the heterotype division. The nucleolus reappears during the telophase, and may be observed in each daughter section of the nucleus, but it does not stain readily, and is not easily recognized (Pl. XVI, Figs. 80, 81).

The spindle configurations of the second type are closely allied to those found in the soma; they differ from the latter merely in the presence of centrosomes, and in the character of the nucleolus which is usually indistinct. The division, occurring as it does after the heterotype, and prior to spore formation, is regarded as the second meiotic or homotype division.

The following stages in the homotype division have been encountered:—

(1) *Homotype nucleus.* (Pl. XVI, Fig. 82.) The nucleus is of the 'wheel' type, but the nucleolus is inconspicuous.

(2) *Prophase.* (Pl. XVI, Figs. 83-4.) These configurations probably represent stages in the formation of the chromosomes.

(3) *Metaphase.* (Pl. XVI, Figs. 85-93.) Figs. 85 and 86 show distinct chromosomes on the spindle. In Figs. 87-93 the chromosomes have more or less completely united to form a continuous equatorial band. The spindle membranes, in Pl. XVI, Figs. 86, 90, and 93, retain a certain amount of stain, rendering the observation of detail very difficult. The elongation of the nucleolus, prior to constriction, is shown in Pl. XVI, Fig. 92. The division of the nucleolus appears to have taken place in Pl. XVI, Fig. 93, but only one indistinct daughter nucleolus was observed.

(4) *Anaphase.* The chromosome band divides to form two daughter bands (Pl. XVI, Fig. 94), and these bands segment to form the daughter chromosomes (Pl. XVI, Fig. 95) during the journey to the poles of the spindle, as in the case of the somatic divisions.

(5) *Telophase*. Pl. XVI, Figs. 96, 97 illustrate the early telophase.

The homotype spindle does not appear to reach the boundary of the nucleus in some cases. Thus in Pl. XVI, Figs. 86, 90, 91, and 93, the spindle is separated from the cytoplasm by a narrow, clear zone, which is bridged by achromatic threads. The variation in size of the spindle is also remarkable.

Divisional configurations which are believed to represent a third mitosis have been observed in the cytoplasm after it has undergone segmentation prior to spore formation. The spindles in such cases are exceedingly small, the long axis being less than one-half the length of the corresponding axis in the smallest homotype spindle. Two spindles in metaphase, each showing three chromosomes, are represented in the figures (Pl. XVI, Figs. 98, 99); and an oblique spindle showing V-shaped chromosomes in Pl. XVI, Fig. 100. The telophase is shown in Pl. XVI, Fig. 101. A third mitosis has been occasionally observed by Cook in *Ligniera*. The spore nucleus is shown in Pl. XVI, Figs. 102 and 103.

VI. DISCUSSION.

The results obtained in this investigation clearly show that Nawaschin was right in assuming that the somatic divisions in *Plasmodiophora* are of an indirect type. All the stages discovered by Nawaschin, and observed five years afterwards by Prowazek, have also been found by the present writer in *Plasmodiophora* and *Spongospora*, and there is no doubt whatever that they represent the more frequently recurring stages in a karyokinetic division. These isolated stages did not include those in which individual chromosomes could be distinguished, and unfortunately the cytologists who have studied the problem since 1899 have attempted to explain the divisions on the scanty information available, instead of making a sustained effort to elucidate them, and for close on thirty years it has been assumed that the chromatin does not condense to form separate chromosomes in the somatic divisions in the Plasmodiophorales.

Prowazek, who possessed an extensive knowledge of the cytology of the Protista, was already familiar with nuclear figures, such as those observed by Vahlkampf in *Amoeba*, which were not at all unlike those recorded for *Plasmodiophora*, and he attached considerable significance to the resemblance. He considered that the nuclear figures observed in *Plasmodiophora* represented various transformations undergone during division of an 'Innenkörper', which he regarded as homologous with the karyosome of the Protista. Unfortunately, the term karyosome has been given different meanings by protozoologists. Prowazek himself at first (49), compared the 'Innenkörper' with the karyosome of *Clossia*. *Clossia* was investigated by Siedlecki in 1898, and his conception of the karyosome, as brought out later in a paper on *Caryotropha* (54), is that it is a structure of

physiological significance, and, as aptly expressed by Dobell (15), 'an amplification of the whole nuclear apparatus'. Prowazek later (50), interpreted the karyosome in terms of the binuclearity hypothesis of Schaudinn. Thus he recognizes two types of chromatin: the chromatic or somatic, functioning chromatin (evident in the macronucleus), and a generative, resting, sexual chromatin (evident in the micronucleus). Maire and Tison ((38) *Sorosphaera*, 1909) follow Prowazek in accepting Schaudinn's hypothesis of the dual nature of the nucleus. The chromatin (phase schizogonique) is entirely concentrated in a nucleoloform karyosome, which at the time of mitosis becomes differentiated into vegetative chromatin (trophochromatin), and generative chromatin (idiochromatin), dividing by direct and indirect methods respectively. The karyosome in a state of rest corresponds to the nuclei of *Trypanosoma noctuae* or *Amoeba limax*, and during division to those of *Caryotropha mesnili*. to the macronucleus of the Infusoria, to the true chromidia of Goldschmidt and, to a certain extent, to the nucleolo-centrosome of *Euglena*.

Osborn (47), who published an account of the cytology of *Spongospora* in 1911, described the vegetative or somatic divisions as amitotic, although the earlier investigators had not abandoned the view that the divisions in the allied genera are of an indirect type. The amitotic effect shown in Osborn's illustrations of the division is due to a very imperfect representation of the nuclear configurations.

Winge¹ (68) devoted special attention to *Sorodiscus*, a parasite so nearly resembling *Sorosphaera*, 'that it would seem most reasonable to unite them into one genus'. Winge deserves credit for observing that, during the somatic divisions, the chromatin often aggregates to form 3-4 bodies in the equatorial plane very similar in appearance to chromosomes. Referring to *Sorosphaera* (68, p. 13) he states, 'the idiochromatin has the form of a plate, though the most chromophilous parts are lying in the periphery of the plate as rather conspicuous grains. The number of these is probable constant, but very difficult to determine on account of their smallness and the surrounding chromophilous substance. In several cases I have observed that their number is not very great'.

Cook ((10), *Ligniera*, 1928) states that the nuclei of the myxamoeba divide by protomitosis, a variety of promitosis, one of the 'five distinct types of primitive mitosis' described by Alexeieff² (1)—see *Sappinia*

¹ The present author wishes to correct a statement made by Winge on a matter introduced into his memoir (68, p. 25) without any obvious reason, viz., 'The author (Horne) has on *Pteris* found an organism in the cellular tissue of the rhizome-apices. According to his description and bad illustrations we have reason to believe that all his "bodies" are oil-globules, precipitation, degeneration, and other phenomena in the tissue of the host-plant.' The author (27) neither found nor described an *organism* in *Pteris*. The material used was properly fixed and the illustrations given in the paper quite fairly represented the structures as observed in the living cells of *Pteris*.

² The significance of the inverted commas in the title of Alexeieff's paper was not borne in on Cook. Alexeieff merely endeavoured to classify the various methods of mitosis shown by the

(*Amoeba*) *diploida*, where 'la chromatine périphérique se trouve à la division disposée d'une façon diffuse entre les deux corps polaires' (1, p. 346). He regards the form of nuclear division recorded by Winge (*Sorodiscus*) as intermediate between protomitosis, and the more complex mitosis found in the higher plants. Cook states that Osborn (*Spongospora*) came to conclusions very similar to his own, adding that the differences between the division in *Spongospora* and *Ligniera* respectively are extraordinarily slight.

The additional stages in the somatic division (*Spongospora*) described in this paper clarify the whole position. Perhaps the most interesting of these stages are those of the previously undiscovered prophase. They include a typical spireme as well as others which show four characteristic V-shaped chromosomes prior to their arrangement on the spindle. These chromosomes take up a horizontal position in relation to the spindle-axis and join to form a continuous band at the equator of the spindle. The band divides to form two daughter bands which segment to form the daughter chromosomes during the journey towards the poles. The behaviour of the chromosomes during the metaphase and anaphase is not strictly in accordance with that usually encountered in nuclei dividing by karyokinesis. The horizontal position taken up during the metaphase is unusual, but perhaps not very significant because even in the higher plants where the chromosomes are relatively large, the chromosomes may at first move into a horizontal position in relation to the spindle as Digby has shown. The end-to-end arrangement of the chromosomes is only possible in cases where $R = 1$ and is not likely to occur in the majority of the plant phyla where the ratio greatly exceeds unity. The nucleolus persists throughout the somatic mitoses without showing any marked decrease in bulk or staining less intensely with iron haematoxylin. It divides by constriction into two daughter nucleoli concurrently with the division of the chromosomes. The cruciform figures which have aroused so much speculation are mainly metaphase configurations viewed from the side.

Cook having assumed that the cruciform configurations represent a stage in a primitive type of nuclear division—protomitosis—proceeds to use this character, i.e. the occurrence of protomitosis, for diagnostic purposes. He claims that it serves to distinguish the Plasmodiophorales from all other fungi and to connect them with the Protista. Since the 'cruciform divisions' must now be regarded as of a karyokinetic nature these views are not so well founded, but the fact remains that the mitoses are of an unusual

Protista. He plainly states his position in the opening paragraph of his paper: 'Le terme de "mitose primitive" par lequel on a désigné la mitose de divers Protistes, est très mauvais et doit disparaître. En effet, d'une part, cette mitose dans la plupart des cas n'est point primitive, et d'autre part ce terme est trop vaste, il englobe des mitoses très variées: les différences, qui séparent ces modes mitotiques, peuvent être presque aussi importantes que les différences entre la mitose et la division directe chez les Métazoaires et les Métaphytes.'

type, and a comparison with the best known cases of nuclear division in some of the chief groups of the lower organisms may not be out of place here.

As a rule the accounts given of indirect division in the fungi are very incomplete, few stages are represented, the chromosomes figured are not sufficiently discrete and their disposition on the spindle is not clearly shown. Comparatively little attention has been given to the dividing nuclei in the vegetative hyphae, interest being chiefly centred around the reproductive organs. The representations of dividing nuclei in *Basidiobolus lacertae* given by Loewenthal ((37) Taf. 11, Figs. 48-64) resemble Nawaschin's figures, except that the nucleolus is not prominent. Others given by Stevens show spindles which recall some of those seen in the homotype divisions in *Spongospora*, for example, in *Albugo Tragopogonis* ((58), Pl. III, Fig. 27), and in the oogonia of *Sclerospora* ((57), Pl. XVII, Fig. 2), in the latter case a nucleus in metaphase is shown with the chromosomes arranged in an irregular band. Miyake (40) shows several karyokinetic figures taken from antheridia and oogonia of *Pythium de Baryanum*, in this case, individual chromosomes are distinctly represented (metaphase). Some of the most detailed accounts of nuclear division have been given by those investigators who have specially interested themselves in the study of sexuality in the Ascomycetes and the events chiefly dealt with are those associated with sexual fusion, fusion in the ascus and the mitoses following this later fusion. Of these mitoses, the first and third have been shown, in several cases, to be reducing divisions—meiotic and brachymeiotic respectively—the second one being the homotype. In such cases, none of the mitoses following sexual fusion are equivalent to those taking place in the soma and to the homotype division in the Plasmodiophorales, where the nuclei are haploid. In *Phyllactinia corylae*, investigated by Harper (25) the homotype, and in this case haploid chromosomes (anaphase) are represented as dots, eight at each pole: in *Pyronema domesticum* the haploid chromosomes in anaphase, seven in number, are similarly represented by Tandy (62): in both cases the condition $R = 1$ is possible since the chromosomes are small in relation to the size of the spindle. According to Fraser (24) in *Humaria rutilans*, sixteen relatively long V-shaped diploid chromosomes are present during the homotype division. Here $R = 4$ approximately, judging from the figures ((24), Pl. XXII, Figs. 35 and 36). In *Laboulbenia chaetophora*, investigated by Faull (20) the primary spore nucleus, in metaphase, shows four strongly curved chromosomes. Here $R = 1$ approximately, but the chromosomes do not straighten out to form a band. A conspicuous nucleolus is present, but it is excentric. A persistent nucleolus is not of uncommon occurrence in the fungi. In *Pustularia bolarioides*, according to Bagchee (2) it is prominent and persists throughout the three divisions in the ascus—see also Hymenomycetes ((63) Wager).

Blackman (3) and Blackman and Fraser (5) have described certain

simplified forms of division in the Uredineae which they consider as reduced from a typical method of karyokinesis. Some of the figures of dividing nuclei given by Blackman (3) Pl. XXII, Figs. 40, 41; Pl. XXIII, Fig. 72) recall various effects observed during mitosis in the Plasmodiophorales.

Němec has observed, in *Sorolpidium betae*, nuclear divisions of two types which closely resemble those recorded in previous work on the Plasmodiophorales, 'Die vegetativen Kernteilungen von *Sorolpidium*, sind ähnlich wie jene von *Plasmodiophora* und *Sorosphaera* durch die Persistenz und Teilung des Karyosoms ausgezeichnet' ((42), p. 49). An illustration of a typical cruciform configuration is given ((43), Taf. II, Fig. 34). Schwartz (53) states that the 'cruciform type of vegetative division' is found in *Olpidium*, but does not show dividing nuclei in his figures. Wager (64) records ten to twelve very minute chromosomes in *Polyphagus*, but their shape and arrangement are not clearly shown. Curtis (11) has given an account of the mitoses in the prosorus of *Synchytrium endobioticum* which result in the formation of the gametes. The chromosomes are unexpectedly minute. In the primary mitoses, 'their form is almost spherical, and they lie close to one another in the equatorial region of the slender-pointed spindle' ((11), Pl. 13, Fig. 41). Judging from the description and figures, the value of R is, in this case, less than unity.

It has been assumed that the anomalous method of nuclear division under discussion is not encountered in the Myxomycetes although comparatively little is known of the nuclear divisions which take place during equivalent developmental stages, i.e. during the multiplication of the myxamoebae, and it is with these early divisions that direct comparison should be made. Olive (44) in following out the nuclear phenomena in the Acrasieae, distinguished two types of nuclear and cell division. The first one of these occurs shortly after spore germination and corresponds to an indirect division. During an early stage in the division he observed an irregular thread of chromatin which suggested a spireme. Next the chromatin granules are aggregated so as to form a group possibly comparable to a nuclear plate. Following this stage a division occurs and subsequent separation of the rod-shaped bodies (chromosomes). Each daughter nucleus retains usually three to four chromosomes. Olive's figures ((44), Pl. VI, Figs. 55, 56, 66-72, and 74) do not show the cruciform appearance simply because a nucleolus is lacking. The second type of division follows after the myxamoebae have had a more or less prolonged vegetative existence. In preparing for the division, the myxamoeba becomes somewhat elongated, and a constriction appears which finally divides the individual into two approximately equal parts, each part receiving one or more of the deeply staining nuclear bodies. The whole process takes place within a few minutes. The figures given by Strasburger (59) of nuclear division in the sporangium in *Trichia fallax* show appearances

which recall the metaphase configurations observed during meiosis in the Plasmodiophorales. Lister's figures are drawn to a larger scale. The chromatin elements comprising the nuclear plate when seen under the highest power appear as elongated curved bodies. The chromosomes both in metaphase and anaphase, in the dividing nucleus of the swarm-cell in *Reticularia Lycoperdon* ((36), Pl. XXXVI, Fig. 1c) are shown in contact, though whether they actually form an annulus is of course uncertain. Again, the absence of the cruciform effect is due to the apparent absence of the nucleolus. The arrangement of the metaphase chromosomes in the amoeba of *Physarum didermoides* appears to be normal from Jahn's figures ((34), Pl. XI, Fig. 1) where eight abbreviated V's are represented.

Swingle's figures of dividing nuclei, in *Stypocaulon*, showing an almost continuous chromosome band ((61), Taf. XVI, Fig. 21) or daughter-bands ((61), Taf. XVI, Fig. 27) are figures of the Nawaschin type with the nucleoli deleted. The same may be said for some of the configurations described by Westbrook, in the Florideae, for example, those observed in the cortical cells of *Rhodomenia palmata* ((65), Pl. II, Figs. 17-19) *Chondria dasyphylla* ((65), Pl. II, Figs. 9-16), and *Laurencia pinnatifida* ((65), Pl. III, Figs. 1-8). The nuclei are exceedingly small ($3.5\ \mu$ in *Laurencia pinnatifida*), and the appearances observed in these cases are attributed to the presence of approximately forty chromosomes banded together—the nuclear membrane and nucleolus vanish prior to the metaphase. Similar figures have been observed by Carter, in the Ulvaceae ((8), Pl. XXII, Figs. 10, 11). Again, the nuclei are very small— $2\ \mu$ in diameter with nucleolus $0.8\ \mu$ increasing to $3.5-4\ \mu$ at the onset of mitosis. The chromosomes are very rarely visible as individual granules. In the lateral view of the metaphase, the chromatin appears as a solid bar—the nucleolus has vanished. The chromosome number, in the Ulvaceae, as estimated, varies from four to ten. In the case of algae with larger nuclei, e.g. *Fucus*, investigated by Strasburger (60) in 1897, the mitoses are less liable to present deceptive appearances.

Dobell (14) has shown that in many groups of Protista it is possible to trace a fairly perfect series of nuclear types from simple amitotic nuclei up to nuclei dividing by a complex mitosis. In 1914 Dobell gave a very detailed account of the cytology of three species of *Amoeba*. Referring to previous work on *Amoeba* he states (16, p. 146) that it would be impossible, in his opinion, 'for anybody who does not possess a first-hand knowledge of a large number of Amoebae to discover, from reading the published accounts, the real facts concerning the division of the nucleus in any single species which has been described'. Dobell describes the nuclear apparatus in terms of a single entity, the karyosome, and that portion of it which is structurally equivalent to the nucleolus in plant organisms, is not clearly

defined in Dobell's figures. The organization of the chromosomes appears to be related to a phenomenon analogous to that described by Curtis as 'nucleolar discharge'. In *Amoeba glebae*, the chromosomes, sixteen in number, are mere spherules measuring only $0.25\ \mu$ in diameter, as determined from configurations in prophase ((16), Taf. 8, Figs. 43, 44). During the metaphase, the groundwork of the karyosome becomes lenticular and then globular with a ring of chromosomes surrounding it in an equatorial fashion and situated just within the boundary of the nucleus. It is almost impossible to distinguish the chromosomes as individuals from this stage onwards. Since the sum of the diameters of the chromosomes is only $4\ \mu$, this feature of the metaphase is somewhat unexpected. The nucleus is from 5.5 to $6.25\ \mu$ in diameter, selecting a nucleus $5.6\ \mu$ in diameter, the circumference is $17.6\ \mu$, or more than four times the sum of the chromosome diameters. Since an increase in width of the chromosome ring is not indicated in Dobell's illustrations ((16), Taf. 8, Figs. 45, 46), it seems probable that the chromosomes are not spherical when they have passed on to the spindle; they may possibly take the form of an abbreviated V, so that an end-to-end arrangement analogous to that found in the Plasmodiophorales is not out of the question. This resemblance is merely conjectural. The mitoses observed by Dobell in *A. lacertae*, *A. glebae* and *A. fluviatilis*, as well as those described by Ford (23) in *A. tachypodia*, differ in other respects from the divisions in the soma in the group in question.

From the selected examples of nuclear division in the Fungi, Algae, and Protista given in the preceding pages it is quite clear that the use of criteria relating to the type of nuclear division is of very doubtful value at the present time in discussing the actual relationship between group and group. The limitations to the power of observing the miniature nucleus are manifest. The divisions, as represented in the three classes of organisms, are often of a more or less generalized type because the actual size, shape and number of the chromosomes is somewhat problematical, and hence they tend to show a general likeness. The resemblance is obviously false in some cases. Thus the chromosome-ring effect may be caused by the more or less close approximation of few V-shaped chromosomes (*Spongospora*), several presumably abbreviated V's (*Amoeba*) or numerous indeterminate chromosomes (Floridaee). Deviations from standard types of mitosis, such as 'normal karyokinesis', are usually recorded in terms of some easily observed character relating to the divisional configurations, and the systematization of nuclear division has perhaps reached an extreme in the Protista (1). Distinct 'types' of nuclear division are sometimes merely variants of one kind of mitosis, as in the Plasmodiophorales, where the divisions in the soma have been accepted as amitotic (47) or protomitotic (10) and the homotype as mitotic, although in reality they are all equivalent mitoses. Finally, an examination of the literature of the Plasmodiophorales

and Protista discloses purely fictitious types of nuclear division. These are based on fragmentary observations by various investigators of isolated stages in division, and comparisons are thereby rendered exceedingly difficult.

The somatic phase ends with the cessation of nuclear division in the soma and then the transitional phase of the life-cycle begins. As consistently recorded by all the workers who have devoted their attention to the cytology of the Plasmodiophorales the transitional phase (akaryote stage) is marked by the presence of relatively large chromidia in the nuclei and in the cytoplasm, and after a time the nuclei lose their affinity for the iron haematoxylin stain. Later on (meiotic phase) chromatic nuclei are again evident, but these do not resemble the characteristic nuclei of the somatic phase. Osborn, referring to *Spongospora*, states that the old somatic nuclei disappear, new nuclei arise later, and later still the 'new' nuclei fuse in pairs. These results are at variance with those obtained by the present author, who finds that the chromatic nuclei observed after the akaryote stage has passed, the 'new' nuclei of Osborn, are diploid, showing eight chromosomes. It is evident therefore that the nuclei unite in pairs *during the transitional phase* and not at its close, but the actual movements leading up to nuclear fusion have not been observed. It is not unlikely that the nuclear unions follow a fusion of coenocytic growth forms of opposite sex as observed by Skupienski (55, 56) in the Myxomycete *Dictyostelium mucoroides*.

No information is available with regard to the early stages in the formation of the fusion nucleus. Observations on the late stages show a departure from the normal fusion process. Fraser (24), describing the fusion of the diploid nuclei in the ascus of *Humaria rutilans*, states that the nuclei enter independently upon the prophase of the first meiotic division, and during the fusion the nuclei lie together against each other in the ascus and appear simply to flow together. In *Trichia* and *Arcyria*, according to Kränzlin, the nuclei prior to fusion are uninteresting because the chromatin bodies cannot be disentangled. She remarks that 'Die Fusionsbilder sprechen für sich'—V-shaped chromosomes and V-shaped chromatic loops in a continuous thread are indicated by the drawings ((35) Taf. 4, Figs. 5 and 6). It is clearly shown that these loops or chromosomes become intermingled during the fusion, and soon after the fusion the nuclei show a chromatic network (unentwirrbares Chromatinnetz) resulting apparently from the union of the haploid chromosomes. The stages found by Kränzlin have not been observed in *Spongospora*, instead, as shown in Pl. XVI, Figs. 56–9, four V-shaped chromosomes develop at each pole of the fusion nucleus and later the eight chromosomes assemble together to form an excentric group situated midway between the poles. These changes indicate that the union of the chromosomes takes place some time after the pairing nuclei have united to form the fusion nucleus. Several

cases of this kind where the fusion is delayed and sometimes greatly protracted have been recorded, for example, in *Amoeba diploidea*, by Hartmann and Nägler (26); in *Laboulbenia chaetophora*, by Faull (20) and in the Uredineae where the fusion process is drawn out through several generations of the synkaryon as Blackman (3) has shown.

Between the nuclear events which have just been considered and the next one observed, which is undoubtedly diakinesis, an apparent hiatus occurs and the missing movements in the cycle of change are those which characterize the prophase of the first meiotic division in animals and plants. For the sake of clearness the salient features of the prophase as recognized by Farmer and Moore ((18) p. 545) will be enumerated.

(1) The chromatin becomes finally arranged in a number of definite loops. When these loops can be counted, there are always found to be half as many loops as there were somatic chromosomes in the preceding premeiotic division.

(2) The whole chromatin network contracts away from the nuclear membrane—first contraction figure.

(3) The loops become increasingly chromatic and open out again—the coarse spireme.

(4) A second contraction and thickening of the individual loops—synapsis.

(5) Each of the individual loops in this second contraction figure becomes directly converted into one of the heterotypic chromosomes.

The cases under review by Farmer and Moore are those in which a long series of diploid nuclear divisions culminate in meiosis. In the case of *Spongospora*, the first meiotic division not only follows nuclear fusion, but actually takes place in the fusion nucleus, and the same phenomenon is also encountered in the Myxomycetes. Kränzlin obtained certain intermediate stages between nuclear fusion and diakinesis in *Arcyria* and *Trichia*. After the stage showing a chromatic network, to which reference has already been made, the nuclei become swollen and stain more intensely. The swollen synaptic nucleus gradually returns to its normal size and then figures are observed which frequently recur in preparations showing rod and V-shaped chromosomes disposed in pairs. Kränzlin's drawings ((35) Taf. 4, Figs. 10, and 11) representing the latter stage strikingly recall the illustrations given by Farmer and Moore of the late stages in the formation of the premeiotic chromosomes in *Periplaneta* ((18) Pl. XXXVIII, Figs. 37, and 48). Kränzlin suggests that the figures show the stage of diakinesis and that such figures indicate that the chromosomes which had united during the synapsis had separated again. The expected division (heterotypic) was not observed. It seems highly probable from the recorded chronological order of the nuclear events that Kränzlin's synaptic con-

figurations represent a true synapsis.¹ Olive (45) in 1907, held the opinion that a true synapsis occurs in *Ceratomyxa*. In the case of *Spongospora* synaptic configurations have not been identified with certainty, although they doubtless occur in the author's preparations.

The cases under consideration (*Arcyria*, *Trichia Spongospora*) are unusual in that karyogamy and the first meiotic division follow one another without any intervening period of 'rest'. This sequence of nuclear events appears to be associated with some modification or abbreviation in the prophase in the first meiotic division as encountered in typical cases of meiosis. The extent of the change in the prophase is uncertain owing to lack of evidence, and further very detailed cytological work on this particular stage in the life-history of the Plasmodiophorales and Myxomycetes is necessary before the matter can be satisfactorily settled.

With great unanimity two consecutive post-translational mitoses have been recorded by the authors who have studied the cytology of the Plasmodiophorales, although in some cases it is not clear from the evidence presented by them that more than one mitosis in time had been actually established. No less than three different explanations of these mitoses have been given by different authors:

(1) They are mitoses to which no significant meaning should be attached—suggested by Prowazek (50), who held the opinion that they were followed by reduction and nuclear fusion.

(2) The second mitosis is the heterotype of a reduction division—advanced by Winge (68), who had not observed nuclear fusion.

(3) The first mitosis is the heterotype—advanced by Maire and Tison (38), Osborn (47), and Cook (10). Osborn holds that the mitoses follow nuclear fusion.

Farmer and Moore (18) applied the term meiotic phase to cover the whole series of nuclear changes included in the two divisions that were designated as heterotypic and homotypic by Flemming, and they explain the essential peculiarities of the meiotic phase as follows: 'They are due to the coherence in pairs of premeiotic chromosomes and to the intercalation of a special form of chromosome—distribution during the course of what would not differ materially from an ordinary premeiotic division'. The various stages in the coherence of the premeiotic chromosomes have been studied in elaborate detail by Farmer and Moore (18), Farmer and Shove (19), and by Digby (13): and the characteristic appearances presented by the chromosomes during and after cohesion are well known and have been observed in organisms differing widely in affinity belonging to both the vegetable and animal kingdoms. The recognition of the occurrence of the

¹ Synapsis, according to Farmer and Moore, 'represents that series of events which are concerned in causing the temporary union in pairs of the premeiotic chromosomes previously to their transverse separation and distribution in their entirety between two daughter nuclei'.

meiotic phase in the Plasmodiophorales should therefore depend either on the identification of the heterotypic chromosome or on some definite stage in the first meiotic division. These conditions have clearly not been satisfied. Reliance has been placed on the so-called 'synaptic' configurations and on the superficial characters presented by the spindle configurations. Maire and Tison's figures of synapsis in *Sorosphaera* ((38) Figs. 36-41) illustrate merely different distributions of chromatic material. The configurations represented were not studied in sufficient detail, and it is not clear whether they are arranged in correct chronological order. Osborn's figures ((47) Pl. XXVII, Figs. 26 and 27) of synapsis and spireme emerging from synapsis are unintelligible.

The interpretation of the spindle configurations depends either on estimates of the chromosome numbers or upon observations relating to the size of the chromatin 'plate'. When it is remembered that one observer after another failed to distinguish chromosomes in the dividing nuclei of the soma, it is surprising to find no less than eight, single or double chromosomes, and even sixteen daughter chromosomes, recorded for the nuclei in metaphase. Winge's Fig. 41 (*Sorodiscus*) which is supposed to represent sixteen chromosomes is possibly a prophase and certainly not a 'side view of the metakinetic plate' shown in vertical view in Fig. 40. Figs. 40 (first sporogonic division) and 45 (second sporogonic and 'heterotype' division), as well as Figs. 68 (*Sorosphaera*) and 74 (*Tetramyxa*), all resemble spindle effects observed by the present writer in *Spongospora* (four chromosomes). Maire and Tison's Figs. 42 and 43 show, according to the authors, heterotypic spindles. Fig. 42, which illustrates 'Fin de la segmentation cytoplasmique et de la formation des chromosomes', shows eleven nuclei. These nuclei show a varying number of dots—usually exceeding ten—which have no very definite shape. Fig. 43 illustrates the metaphase, and nuclei are represented viewed from above and from the side: the former show more or less V-shaped or looped chromosomes, and it is obviously intended to represent eight. These figures again recall effects seen in *Spongospora*, as does also Osborn's Fig. 32 showing 'eight' chromosomes in equatorial view. The chromosome number, eight, dates curiously enough from the work of Jahn (31-4) and Kränzlin on the Myxomycetes. Cook ((10) p. 359) states,¹ 'It will be seen that the size of both the spindle and of the metaphase plate in the second division is about half the size of that found in the first division. The conclusion which must necessarily be drawn from such a figure is that the first division in the zoosporangium is meiotic, and that the quantity of chromatic material present in the second nuclear division is half what was present in the first'.

¹ The following statement occurs in the same paper ((10), p. 357): 'It is therefore impossible in the case of *Ligniera junci* to bring forward any conclusive evidence that these represent a heterotypic and homotypic division'.

The evidence presented in this paper in favour of meiosis in this group is related to the number and shape of the chromosomes as observed chiefly in *Spongospora*. Four V-shaped chromosomes are present in the somatic, haploid nuclei and eight V-shaped chromosomes in the diploid nuclei. The first meiotic division has been recognized owing to the occurrence of diakinesis and of heterotype chromosomes of characteristic forms. The first meiotic division is followed by one or more mitoses, in which again four V-shaped chromosomes are evident. The failure on the part of the previous authors to establish meiosis is due to the fact that they were unacquainted with the different forms assumed by the chromosomes and were accordingly unable to distinguish between somatic and heterotypic chromosomes. They had obtained no guidance from the divisions in the soma, having assumed that the chromatin does not condense to form separate chromosomes in the dividing nuclei. For these reasons the chromosome numbers present during the post-transitional mitoses could not be accurately determined and the recognition of specific mitoses presented insuperable difficulties.

VII. SUMMARY.

1. Existing accounts of the life-history of the Plasmodiophorales are unsatisfactory. The divisions in the soma have been consistently misinterpreted, and the occurrence of meiosis has not been proved.

Somatic Phase.

2. The nuclei divide by karyokinesis. They are haploid and the chromosome number is four.

3. The chromosomes in metaphase become united end-to-end to form a continuous band encircling the nucleolus. The band divides into two daughter bands which break up to form the daughter chromosomes during the journey to the poles. The nucleolus is prominent and persists throughout the somatic phase.

Transitional Phase.

4. At the close of the somatic phase the nuclei become achromatic. Concurrently the cytoplasm stains more intensely owing to the presence of numerous chromidia.

5. It is assumed that a fusion of coenocytic growth-forms followed by the union in pairs of nuclei of opposite sex takes place during the transitional phase.

Meiotic Phase.

6. The post-transitional nuclei show well-developed asters and centrosomes. The nucleolus is inconspicuous.

7. The post-transitional nuclei are diploid. Four chromosomes are

organized in the neighbourhood of each pole. The nucleolus vanishes. The two groups of chromosomes assemble together at one side of the nucleus, producing thereby the effect of contraction.

8. The next stage observed is that of diakinesis. The diakinetid figure shows four chromosomes, of which one is ring-shaped.

9. The heterotype spindle configurations show four chromosomes. The chromosomes in metaphase and in the diaster assume forms similar to those described by Farmer and Moore and many others as characteristic of the heterotype division in animals and plants. The nucleolus reappears in the telophase.

10. The homotype division resembles the equivalent somatic mitosis except that the nucleolus is very inconspicuous. The spindles vary greatly in size.

11. The meiotic divisions are sometimes followed by a third mitosis which immediately precedes spore formation.

12. It is considered premature to discuss the affinities of the Plasmodiophorales in terms of nuclear structure and changes for the following reasons:

(a) There is a dearth of accurate detailed information with regard to the structure of the nucleus and its behaviour during mitosis in the more lowly organized groups of animals and plants.

(b) Such resemblances as there are, in the nuclear phenomena, between group and group do not necessarily indicate relationship: they may be due merely to parallel development.

In conclusion, the author's thanks are due to Professor Sir John Farmer for his very valuable help and criticism.

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EXPLANATION OF PLATES XV-XVI.

PLATE XV.

- Fig. 1. *Spongospora*. Somatic nucleus showing nucleolus, linin 'threads', chromatin 'granules', and the nuclear membrane.
 Fig. 2. *Sorosphaera*. Somatic nucleus showing asters and centrosomes.
 Fig. 3. *Entamoeba histolitica*. An amoeba showing the alveolar nucleus. Dimensions: amoeba, $7.2\ \mu$; nucleus, $2.7\ \mu$; nucleolus, $0.6\ \mu$ in diameter.
 Fig. 4. *Entamoeba histolitica*. Another amoeba, $18\ \mu$ in length; nucleus, $6\ \mu$ in diameter.
 Fig. 5. *Entamoeba coli*. Amoeba showing the alveolar nucleus. Dimensions: amoeba, $30.6\ \mu$ in length; nucleus, $7\ \mu$; nucleolus, $1.2\ \mu$ in diameter.
 Fig. 6. Miniature yeast showing nuclei of the 'wheel' type. Nucleus, $3.6\ \mu$; nucleolus, $1.2\ \mu$ in diameter.
 Figs. 7-18. *Spongospora*. Prophase of the somatic mitosis.
 Figs. 7-10. Earliest stages—formation of chromatin 'rods'.
 Figs. 11, 12. Showing tendency to form a chromatin network.
 Fig. 13. Early spireme stage.
 Fig. 14. Complete spireme.
 Figs. 15-18. Late stages in the formation of the somatic chromosomes.
 Figs. 19-32. *Spongospora*. Metaphase of the somatic mitosis.
 Fig. 19. Chromosomes prior to the formation of the spindle.
 Fig. 20. Two chromosomes normally and one inversely orientated. Distinct spindle fibres present. Spherical nucleolus.
 Fig. 21. Three chromosomes, normally orientated, the fourth not in view. Nucleolus almost cylindrical.
 Fig. 22. Three chromosomes are shown and distinct spindle fibres.
 Fig. 23. Three chromosomes without distinct spindle fibres, the linin 'threads' are conspicuous.
 Fig. 24. Unipolar spindle showing distinct fibres. The chromosomes appear to be inversely orientated.
 Fig. 25. Two chromosomes missing, or each lateral V may consist of two joined end to end.
 Fig. 26. The chromosomes appear to be joined end to end, but do not form a circular band.
 Fig. 27. Unipolar spindle. The chromosomes form a continuous band encircling the nucleolus. Linin 'threads' are also present.
 Figs. 28, 29. Chromosomes united, linin 'threads' distinct.
 Fig. 30. Chromosomes united, linin 'threads' and spindle fibres distinct.
 Fig. 31. Chromosomes united, nucleolus cylindrical.
 Fig. 32. Chromosomes united, nucleolus constricted.
 Figs. 33-44. *Spongospora*. Anaphase of the somatic mitosis.
 Figs. 33, 34. The division of the chromosome band has taken place. The daughter bands have already segmented to form the daughter chromosomes. The nucleolus is spherical, and distinct linin threads are present.
 Fig. 35. The daughter bands are farther apart. The nucleolus is cylindrical.
 Figs. 36, 37. The daughter bands are almost continuous. The nucleoli are cylindrical.
 Fig. 38. The individual daughter chromosomes are distinct.
 Fig. 39. The segmentation of the daughter bands is incomplete. The nucleolus is cylindrical.
 Fig. 40. Six distinct daughter chromosomes are present, the remaining two are missing. The nucleolus is spherical.

Fig. 41. Another example of the same type. The V-shaped upper chromosomes are remarkably distinct.

Figs. 42-4. Examples of nuclei where each daughter chromosome band at first breaks into two portions. The nucleoli are cylindrical.

Figs. 45-9. *Spongospora*. Telophase of the somatic mitosis.

Fig. 45. Six daughter chromosomes are present, three near each pole. The nucleolus has divided.

Fig. 46. Another figure of this type. At least six chromosomes are present.

Figs. 47, 48. Three chromosomes at the upper and two at the lower end of the nucleus are shown. The nucleolus is elongated and constricted.

Fig. 49. The daughter nucleoli are formed, and are surrounded by the daughter chromosomes, of which one is missing in the upper portion of the figure.

Figs. 50-53. *Spongospora*. Transitional phase.

Figs. 50, 51. Early akaryote stage. The nucleolus is less distinct, and the chromidia are larger.

Fig. 52. Chromidia in the neighbourhood of the nucleolus.

Fig. 53. Later akaryote stage. The nucleolus is not easily distinguishable. The chromidia are prominent.

PLATE XVI.

Fig. 54. *Spongospora*. Showing the effect of staining the parasite with fuchsin when in the akaryote condition.

Figs. 55-69. Pre-synaptic configurations.

Fig. 55. Post-translational nucleus showing nucleolus and short polar radiations.

Fig. 56. Nucleus showing chromatin 'granules' at the poles.

Fig. 57. Nucleus showing distinct centrospheres.

Fig. 58. The bipolar chromatin 'granules' are more distinct.

Fig. 59. A looped arrangement of the linin and chromatin is evident.

Fig. 60. The chromatic portions of the loops are more distinct.

Fig. 61. The chromatic portions of the loops resemble chromosomes.

Figs. 62, 63. Nuclei viewed obliquely showing polar groups of chromosomes. The upper group consists of four V-shaped chromosomes.

Fig. 64. Nucleus viewed obliquely showing six chromosomes.

Fig. 65. Nucleus viewed slightly obliquely. Four very distinct V-shaped chromosomes are present at the upper end, but only two were observed at the lower end of the nucleus. The nucleolus has vanished.

Fig. 66. The chromosomes are commencing to assemble.

Fig. 67. Another nucleus showing the same feature. The eight chromosomes are distinct. The centrospheres and centrosomes are not opposite one another.

Fig. 68. Another nucleus showing a similar stage.

Fig. 69. Contraction figure due to the assembling together of the diploid chromosomes.

Figs. 70-81. First meiotic division.

Fig. 70. Diakinesis.

Figs. 71-3. Metaphase showing spindle fibres, centrosomes, and U-shaped chromosomes.

Fig. 74. Metaphase, chromosomes commencing to divide.

Figs. 75-9. Anaphase, showing centrospheres, centrosomes, spindle fibres, and the characteristic heterotype daughter chromosomes.

Figs. 80-1. Telophase.

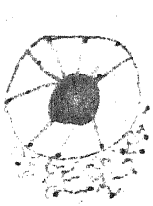
Figs. 82-97. Second meiotic division.

Figs. 82-4. Nuclei in prophase.

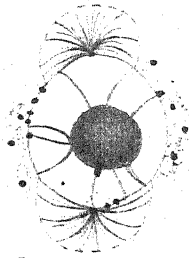
Fig. 85. Early metaphase. Three distinct chromosomes are present, the fourth is missing. The nucleolus is indistinct.

Fig. 86. A similar stage. A second boundary line—nuclear membrane?—is present outside the spindle membrane.

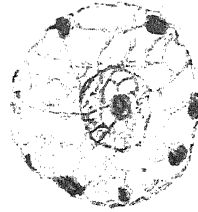
Fig. 87. Metaphase. The chromosomes are continuous, nucleolus very small.



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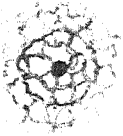
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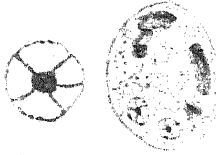
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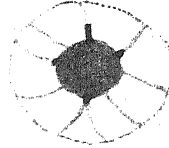
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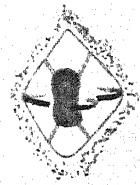
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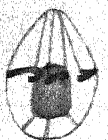
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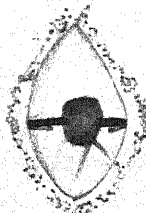
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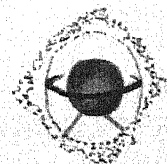
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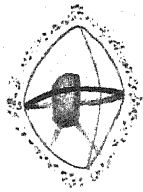
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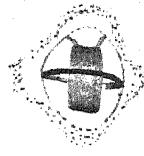
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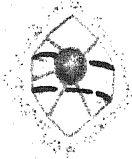
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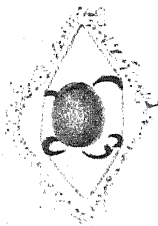
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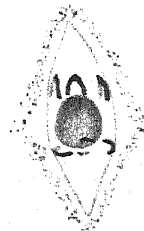
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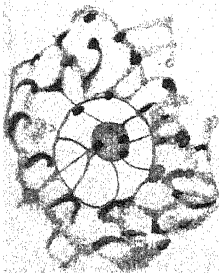
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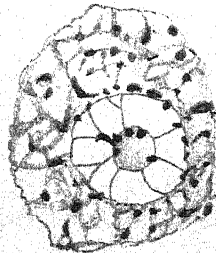
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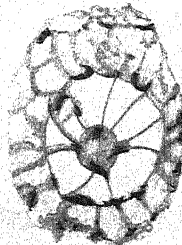
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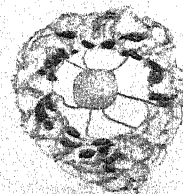
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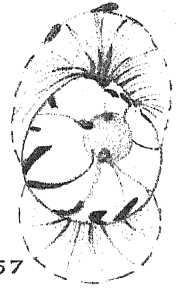
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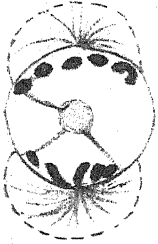
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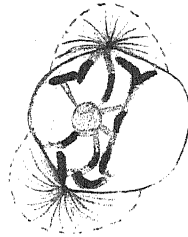
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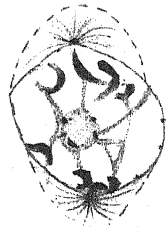
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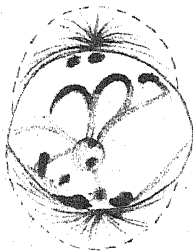
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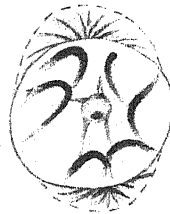
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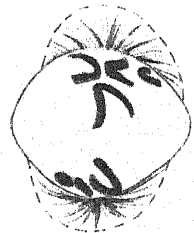
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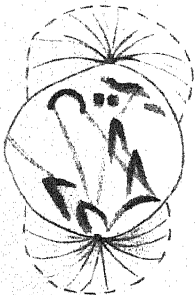
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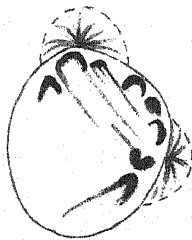
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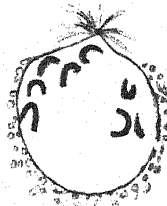
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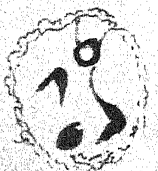
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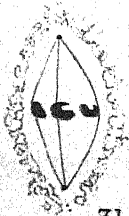
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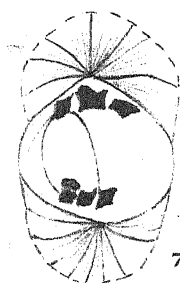
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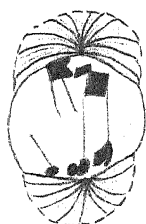
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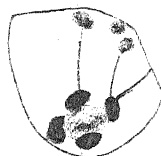
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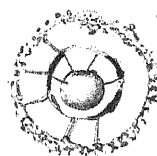
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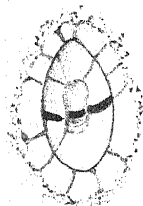
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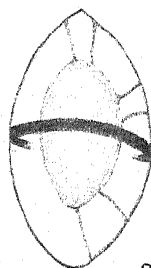
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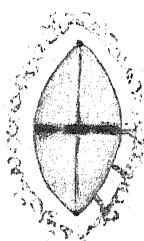
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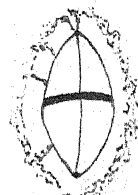
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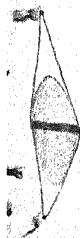
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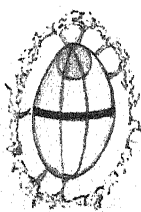
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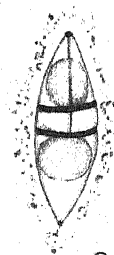
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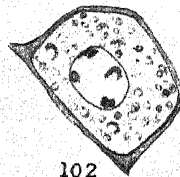
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100



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102



103

- Fig. 88. Metaphase. Another nucleus showing the same stage.
- Fig. 89. Metaphase. Noteworthy for the large nucleus and nucleolus.
- Fig. 90. Metaphase, showing dark spindle membranes. The presence or absence of the nucleolus could not be determined.
- Fig. 91. Metaphase. Outer membrane present. Nucleolus absent.
- Fig. 92. Metaphase. Showing elongated, indistinct nucleolus.
- Fig. 93. Metaphase. The nucleolus has divided, but only one of the daughter nucleoli is distinguishable owing to the darkening of the spindle membrane.
- Fig. 94. Anaphase. The chromosome band has divided to form two daughter bands.
- Fig. 95. Anaphase. The homotype daughter chromosomes are shown.
- Fig. 96. Telophase. Daughter nucleoli present.
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- Figs. 98-101. Third nuclear division.
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- Fig. 101. Telophase.
- Figs. 102, 103. The spore nucleus.

Studies on the Transport of Nitrogenous Substances in the Cotton Plant.¹

IV. The Interpretation of the Effects of Ringing, with Special Reference to the Lability of the Nitrogen Compounds of the Bark.

BY

E. J. MASKELL

AND

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With two Figures in the Text.

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¹ Paper No. 6 from the Physiological Department of the Cotton Research Station, Trinidad.

SECTION I. INTRODUCTION.

IT was concluded as a result of our studies on the transport of carbohydrates (5), that sucrose was the chief form in which carbohydrates were translocated longitudinally through the bark. This conclusion was based on a number of converging lines of evidence, which may be summarized as follows:

1. *Diurnal variation.* The diurnal variation in carbohydrate content of the bark is almost entirely due to sucrose.

2. *Response to ringing.* Sucrose accumulates in the bark above a ring, and diminishes below it, to a far greater extent than reducing sugars.

3. *Vertical gradients.* The vertical gradient of sugar concentration in the bark is very largely a gradient of sucrose, and is approximately proportional to the rate of transport.

4. *Radial distribution of sugars in the bark.* Sucrose shows high concentrations in the inner zone, which contains most of the sieve-tubes, and low concentrations in the middle and outer zones, which are composed mainly of ray tissues and cortex. The radial distribution of reducing sugars is the exact reverse.

5. *Localization of the response to ringing.* The response of sucrose to ringing is mainly a response in the region of the sieve-tubes.

The convergence of these lines of evidence, and, in particular, the localization of the sucrose response to that zone of the bark which, on structural grounds, seems best adapted to transport, renders it probable that sucrose was the chief form in which carbohydrates were transported longitudinally in the plants used in our experiments. To the evidence summarized above we may add also the observation, made during subsequent work (4), that when the direction of movement of carbohydrates in the stem is reversed, the principal change in the sugar gradients is a reversal of the sucrose gradient in the inner half of the bark. It will be evident that identification of sucrose as the principal translocatory compound was only possible because the rate of longitudinal transport was rapid, compared with the rate of conversion to reducing sugars or polysaccharides, and with the rate of radial spread out of the sieve-tubes.

Much of the evidence for the identification of sucrose as the main mobile form depends on the conception of transport as determined by concentration gradients. At the same time the evidence obtained reinforces that conception. The methods adopted and the arguments involved in seeking to identify the mobile form for nitrogen transport must similarly be dependent on some conception of the mechanism of nitrogen transport. We will therefore summarize what appears to us to be the evidence in favour of a gradient basis.

There is first of all the spread of diurnal changes in nitrogen content from leaf to bark (cf. 2). Secondly, there is the reversal of the normal direction of transport in the stem and the gradient changes which accompany this reversal (cf. 4). Thirdly, there is, on ringing, the accumulation of nitrogen not only in bark and wood, but also in the leaves some feet above the ring. The details of this experiment are given in the present paper. Finally, there is the type of response shown to partial ringing (cf. 2).

Some evidence bearing on the identification of the mobile form has been presented already (3, 4). It will be convenient to summarize this under the following heads: (1) radial distribution of nitrogen compounds in the bark; (2) vertical gradients during normal downward movement; (3) change in gradients when the direction of movement is reversed.

(1) *Radial distribution of nitrogen compounds in the bark.* Data obtained by subdividing a specified region of bark into outer, middle, and inner zones (cf. 3), indicate the following distributions of compounds as between sieve-tube tissues (including companion cells), ray tissues, and cortex:

Protein N: no consistent concentration differences, but in general the ratio of protein N to total N lowest in the sieve-tubes.

Total crystalloid N: concentration high in sieve-tubes, low in cortex, intermediate in rays. This would suggest that crystalloid rather than protein N is concerned in transport. The organic crystalloid fractions fall into two classes with characteristic distributions.

Amino-acids and *residual N*, like sucrose, have high concentrations in the sieve-tubes, and low concentrations in rays and cortex. For *asparagine*, on the other hand, the concentration in the sieve-tubes, though much greater than in the cortex, is less than in the rays. This suggests that amino-acids and residual N are concerned with transport and asparagine mainly with storage. Protein N also may be mainly concerned with storage. The evidence in favour of amino-acids is somewhat discounted by their low concentration; even in the sieve-tubes they form only about 14 per cent. of the total crystalloid N. It is quite possible, however, that the figure for amino-acids is an underestimate (cf. 3). Again, although asparagine is present in highest concentration in the rays it still forms about half the crystalloid N in the sieve-tube, so that a transport function for asparagine can hardly be excluded.

(2) *Vertical gradients during normal downward transport.* On the gradient theory of transport, the translocatory compound should show a gradient in the direction of movement. But both total N and crystalloid N show marked negative gradients in the bark. The crystalloid N gradient is due mainly to asparagine, but partly also to amino-acids. The only indication found of a positive gradient was in the residual N fraction, but the small gradients observed were not statistically significant. The case

for residual N as the translocatory fraction is, however, strengthened somewhat by the fact that there is a steep gradient of residual N out of the leaf parenchyma into midrib and petiole. Amino-acids show a similar, though much smaller gradient, but the asparagine gradient is in the opposite direction. It is noteworthy also that, whereas the proportion of residual N and, to a less extent, the proportion of amino-acids tend to decrease as we pass down the stem, the proportion of asparagine increases. As far as it goes, this is in favour of residual N or amino-acids, and against asparagine, being concerned in transport, but the absence of positive gradients in residual N and amino-acids in the bark is a serious difficulty. It may be that definite positive gradients are present in the sieve-tubes, but are masked by negative gradients in the other tissues of the bark. Examination of the vertical gradients in inner and outer halves of the bark showed, however, that the negative gradients of asparagine and amino-acids are somewhat greater in the inner half, which contains most of the sieve-tubes. Also, the small positive gradient of residual N is no more definite in the inner half than in the outer. Subdivision into two zones only does not, however, effectively separate sieve-tubes from ray tissues, and if there were a steep negative gradient in the rays the gradient in the sieve-tubes of the inner zone would still be masked. Consequently the possibility of positive gradients in the sieve-tubes remains open. Masking by gradients in the rays is perhaps more probable for asparagine than for amino-acids, or residual N, since, as we have seen, the concentration of the two latter fractions is very similar in rays and cortex, while asparagine has a much higher concentration in the rays.

(3) *Change in gradients when movement is reversed.* When normal downward movement of nitrogen between two regions of stem is reversed (cf. 4), the vertical gradient of total N, initially negative, becomes still more strongly negative. Eliminating the static component of the observed gradients, by comparing the sum of the concentrations in the supplying regions with the sum of the concentrations in the receiving regions, we find that the dynamic gradient of total N is positive, and is composed mainly of protein N and amino-acid N. There is also a small positive dynamic gradient of asparagine, but, judged from the relation between sugar gradient and carbohydrate movement, this gradient is too small to account for the nitrogen movement observed. The dynamic gradient in amino-acids, however, would appear to be adequate. For the residual N fraction, on the other hand, the data indicate a negative dynamic gradient. The evidence is unfortunately not conclusive, for nitrates were not determined separately but were included with residual N.

To sum up the evidence so far presented, it would appear that each of the nitrogen fractions considered has some claim to be considered the translocatory form, but that there are also serious objections to each fraction.

In favour of *amino-acids*, which would seem on the whole the most probable form, we have the characteristic radial distribution and the adequate dynamic gradient in the Reversal experiment (4): against amino-acids is the apparent negative gradient in the sieve-tube region during normal downward movement. This, however, might be due to negative gradients in the companion cells, or even to a static component in the sieve-tubes themselves. In favour of *asparagine* is the fact that it forms roughly half the crystalloid N in the sieve-tubes; against asparagine is the still higher concentration in the rays, which suggests storage, also the marked negative gradients in the inner half of the bark, and the very small dynamic gradient in the Reversal experiment. It is true that the high concentration in the rays makes the masking of the sieve-tube gradients by ray gradients more probable for asparagine than for amino-acid or residual N, but in that case asparagine should have shown a marked positive dynamic gradient in the Reversal experiment. In favour of *residual N* we have its radial distribution, some slight evidence of positive gradients in the bark during normal downward movement, and also the gradients out of the leaf parenchyma, which suggest that residual N might be the head for transport throughout the plant; against residual N, as against amino-acids, is the failure of the inner half of the bark to show a definite positive gradient, but the most serious objection to residual N is the apparent dynamic gradient in opposition to the direction of movement in the Reversal experiment.

As regards *protein N*, the lower proportion found in the sieve-tubes suggests that it is less important than crystalloid N for transport. Part of the protein N of the sieve-tube is of course cytoplasmic protein, and forms a permanent feature of the structure. But the protein that fluctuates in response to changes in supply or demand must be regarded as labile protein and is presumably either temporary storage protein or actually mobile protein. The positive dynamic gradient of protein N obtained in the Reversal experiment is of course due to this labile protein, and it suggests that some of this protein may be active in translocation. The presence of sieve-pores is in harmony with the view that some movement of colloid takes place, and there seems no reason why the movement of protein *along the sieve-tube* should not be accelerated in the same way as the movement of sugars and other crystalloid substances.

It will be noticed that we have not, as yet, made use of ringing as a means of identifying the mobile compound, and that we have not, as yet, considered the diurnal changes in the nitrogen fractions of the bark. The latter will be considered in a subsequent paper. In the present one attention is focused on the ringing method, which proved so useful in the identification of the mobile form of carbohydrate. Interpretation of ringing experiments is much more difficult in the case of nitrogen than in the case of carbohydrates, for conversion of one form of nitrogen into another may

be relatively rapid in the bark tissues. How extensive this conversion may be is shown by an experiment described earlier ((4), Exp. 7), in which the leaves were removed from one group of plants and the nitrogen of the bark determined after intervals of five and eight days. It was found that, though the total N had not changed appreciably, yet the crystalloid fraction had increased from 25 per cent. of the total N up to 40 per cent. In an experiment described in the present paper much more rapid changes in the proportions of protein N and crystalloid N are shown to occur without any appreciable loss or gain of total N. In the bark interconversion of protein and crystalloid N appears to be very rapid compared with the rate of longitudinal transport. Consequently crystalloid N might move along the bark but accumulate above a ring either as crystalloid N or protein, depending on the conditions determining interconversion. Conversely protein might move down, but accumulate either as protein or as crystalloid N. Interpretation of results will depend, therefore, on our having some information as to the probable direction of the conversion (whether crystalloid N to protein, or protein to crystalloid N) under the particular conditions of the experiments. Clearly some knowledge of the factors determining the conversion of crystalloid N to protein N, and vice versa, is a first essential to disentangling the part played by each in transport. Before, therefore, considering what is the effect of ringing we shall present and discuss the evidence we have obtained concerning some of the factors responsible for the lability of protein in the bark.

The methods employed in determining the different forms of nitrogen have been fully detailed in paper II of this series (3).

SECTION 2. FLUCTUATIONS IN THE RATIO OF CRYSTALLOID N TO PROTEIN N.

Our estimate of the amount of protein N depends on (1) the total N determined on the dried material, and (2) the concentration (per 100 grm. water) of crystalloid N in the expressed sap. The weight of crystalloid N in the sample ($= \text{concentration} \times \text{weight of water in the sample} \div 100$) is deducted from the weight of total N to give the weight of protein N in the sample. As total N and crystalloid N are independent determinations, and protein N contains the errors of both, it seemed preferable to express the relation between the two fractions by stating crystalloid N as a per cent. of the total N rather than by stating the ratio of protein to crystalloid N.

A survey of all our experiments on nitrogen transport in 1927-8 leads to the following figures. For the Lower region of bark the average proportion of crystalloid N (10 experiments) is 40.12. In five of these experiments an Upper region of bark was sampled at the same time. The Upper region in these cases had a consistently lower proportion of crystal-

loid N—the mean difference being 5.13 with standard deviation 0.75. The values obtained for both regions show considerable variation from one experiment to another, and even within a single experiment. In the Lower region the mean values for different experiments cover a range from 25 up to 49, while in one experiment the values obtained may range from 26 up to 46. It will be evident, from the fairly consistent difference between Upper and Lower regions, that the Upper shows a very similar range to the Lower.

(a) *The Effect of Sugar and Hydrogen Ion Concentration on the Ratio in the Bark (Experiments 7 and 9).*

Experiment 7.

In this experiment, as already described (4), removal of the leaves resulted in a large increase in the proportion of crystalloid N in the bark and a parallel decrease in the sugar concentration. There were three groups of plants: (1) Leaves-on group: bearing leaves but ringed at the base of the stem; (2) Leaves-off group: without leaves and similarly ringed; and (3) a Normal group of untreated plants. An initial collection was made from the Normal group on the day of ringing. Collections of Leaves-on and Leaves-off plants were made 5 and 8 days later. In all, including samples from Upper and Lower regions of the stem, there were 24 samples of bark. For these 24 cases the correlation coefficient between total sugar concentration and the proportion of crystalloid N amounts to -0.892 . In 14 cases data are available for the concentrations of sucrose and of reducing sugars. The correlations in this series of 14 are, with sucrose $r = -0.930$, with reducing sugars $r = -0.717$. The regression equation for the correlation with total sugar concentration is: Proportion of crystalloid N = $30.3 - 4.44$ (T. S. -4.46), where T. S. is the total sugar concentration, 30.3 is the mean proportion of crystalloid N, 4.44 is the regression coefficient, and 4.46 is the mean concentration of total sugars. The mean proportion of crystalloid N in the Upper region of the stem was 27.1, and in the Lower 33.5. Part of this difference may be attributed to the higher concentration of total sugars in the Upper region, but only part, for calculation from the regression equation, using the mean sugar concentrations for Upper and Lower regions respectively (viz. Upper 4.709, Lower 4.206), gives the expected values for proportion of crystalloid N as 29.2 Upper region, and 31.4 Lower region. Thus difference in vertical level *per se* seems to determine a different ratio of protein to crystalloid N.

The bark of the Lower region was subdivided into inner and outer halves. The halves show a characteristic difference in proportion of crystalloid N, but within each half there is still the strong negative correlation with sugar concentration which was shown in the bark as a whole. The

correlation coefficients and regression equations for the twelve cases available are shown below :

Inner Half $r = -0.983$. Proportion of Crystalloid N = $40.23-4.23$ (T.S.-4.46).
Outer Half $r = -0.879$. " " = $27.13-4.02$ (T.S.-4.01).

The slope of the regression line is very similar in the two halves, but the inner half has a much higher proportion of crystalloid N, in spite of a somewhat higher concentration of total sugars. It will be seen that the difference in proportion of crystalloid N between inner and outer halves is much greater than the difference between Upper and Lower regions, conforming to the general rule that radial gradients in the bark are much steeper than vertical gradients.

The variation in sugar concentration, and in proportion of crystalloid N, was greater in this experiment than in any other. The experiment lasted, moreover, some days, so that there was time for the adjustment of equilibrium. An experiment of shorter duration, and showing smaller changes, will now be considered.

Experiment 9

This experiment, which was also reported in our previous paper (4), was carried out in order to ascertain whether the normal direction of transport in the stem could be reversed. The mean proportion of crystalloid N was 32.57, a little higher than in Experiment 7, but the variation was much less, a standard deviation of only 3.09 as against 8.55. The correlations found with total sugar concentrations were lower, but in the same direction as before. The sixteen samples available included Upper and Lower regions of bark; half the regions were bearing leaves (supplying regions), and half were leafless (receiving regions). The correlation coefficients and regression equations are shown below.

Whole Bark: $r = -0.746$.	Proportion of Crystalloid	$N = 32.57 - 3.3$ (T. S. -5.77).
Inner Bark: $r = -0.716$.	" "	$N = 38.68 - 3.49$ (T. S. -5.98).
Outer Bark: $r = -0.703$.	" "	$N = 27.11 - 2.68$ (T. S. -5.59).

Inner and Outer halves show the same difference as in the previous experiment.

The regression lines (for the whole bark) for this experiment and for Experiment 7 are shown graphically in Fig. 1 (p. 249). It will be noticed that the regression line in Experiment 9 is not as steep as that of Experiment 7, possibly because the former only lasted two days, so that the equilibrium was not established. But the difference in *level* of the regression line in the two experiments suggests that other factors in addition to sugar concentration, and vertical or radial position, influence the protein-crystalloid N ratio in the bark.

As the concentration of hydrogen ions was determined on most of the

samples in the two experiments just discussed, it is possible to consider its relation to the protein-crystalloid N ratio. The correlation coefficients between the proportion of crystalloid N and the pH are shown below:

	Experiment 7. (16 cases.)	Experiment 9. (16 cases.)
Mean proportion of Crystalloid N .	33.17	32.57
Mean Total Sugar Concentration .	4.15	5.77
Mean pH	6.17	6.29
Correlation Coefficients	r_{12}	-0.951
	r_{13}	-0.605
	r_{23}	$+0.715$
[1 = Proportion of Crystalloid N]	$r_{12.3}$	$+0.931$
[2 = Total Sugar Concentration]	$r_{13.2}$	-0.766
[3 = pH]	$+0.347$	$+0.260$

In Experiment 7 the direct correlation with pH is fairly high and negative, but in Experiment 9 it is small and positive. The partial correlations with total sugar concentration are as high as the direct correlations, but the partial correlations with pH are very small. They are, however, of the same sign, and of the same order in the two experiments, and they may be due to some real effect of pH on the protein-crystalloid N ratio. The data do not, however, establish such an effect. Moreover, the small difference in pH between experiments 7 and 9 will not account for the higher proportion of crystalloid N (at equivalent concentrations of total sugar) in experiment 9 (cf. Fig. 1).

It seems clear from these two experiments that changes in sugar concentration in the bark produced by ringing the stem, or by removal of the leaves, are closely associated with changes in the proportion of crystalloid N in the bark, the proportion of crystalloid N increasing as the sugar concentration diminishes, and vice versa. The different average values of the proportion of crystalloid N in the two experiments are not, however, to be explained by differences in total sugar concentration.

(b) *Rapid Changes of Ratio in the Bark (Experiments 3, 4, and 5).*

That factors other than sugar concentration are at work is also indicated by the fact that sometimes marked changes in the proportion of crystalloid N may occur without any appreciable change in the concentration of sugar. Thus in experiment 3, in which samples of bark were taken from a low region of stem in two groups of plants, one of which had been ringed *below* and the other *above* the region sampled, the proportion of crystalloid N was not much affected by the position of the ring, but in both groups the proportion of crystalloid N altered within a period of three hours from a mean value of 54.89 to a mean of 39.77. It will be noted that the final figure is close to the average value (40.12) for the Lower region of the stem for all the experiments, while the initial values are abnormally high. All other observed cases of rapid changes in the proportion of crystalloid N during

the day were of this type, i. e. a rapid fall from an abnormally high value to one close to the average. The factors responsible for the production of such high values are not known, but the rapid fall may perhaps be regarded as an adjustment to equilibrium conditions.

The results for the three experiments, 3, 4, and 5, in which rapid changes of ratio occurred during the course of the day, are shown in Fig. 1 (p. 249). The arrows show the direction of the change in time. In all cases the change is a fall towards the mean value for all the experiments, which is shown by the horizontal line in the graph. It is remarkable that, in the three experiments in which rapid adjustments in ratio occurred, the changes should all have been in the same direction, i. e. crystalloid N to protein N.

(c) *The Effect of Moisture Conditions on the Ratio in Bark and Wood*
(Experiment 11).

In order to study the effect of changes in moisture content on the protein-crystalloid N ratio, stems were cut into four quadrants by longitudinal cuts. One quadrant was placed in a moist and one in a dry atmosphere, while the other two were used to determine the conditions at the start of the experiment.

Procedure.

The plants were graded by means of circumference measurements on the region of the stem to be sampled. Two samples, each of forty-six plants, were then drawn. From each plant a section of bare stem, 18 in. in length, between the foliage region and the ground, was taken. In the laboratory it was cut transversely into three pieces, each 5 in. in length, and two shorter pieces. The 5-in. pieces were divided longitudinally into quadrants; one of the shorter lengths, taken from near the middle of the 18-in. length, was kept for microscopical examination of the bark. Of the four quadrants, two were taken for immediate analysis, one was put into a dry, and one into a moist chamber. Each of the samples of forty-six stems thus provided a set of equivalent quadrants for each of the following groups—Normal 1 and Normal 2, Moist and Dry.

The moist chamber was a porcelain jar through which air saturated with water vapour was drawn. The dry chamber was a similar jar through which dry air was drawn. The volume of air drawn was not, however, sufficient to produce appreciable drying of the samples, and after a night in this chamber the samples were taken out and spread on the surface of a table. The sequence of events was as follows:

February 14, 12.45 p.m. Collections of A and B samples.

„ „ 1.15 p.m. Subdivision into quadrants. Weighing of each set of quadrants.

- February 14, 2.30 p.m. Quadrants put into moist and dry chambers.
Normal quadrants sampled for analysis.
„ 15, 8.50 a.m. Quadrants from dry chamber spread on table.
„ „ 1.40 p.m. Moist and dry quadrants weighed and
sampled for analysis.

As soon as the sets of quadrants had been weighed, the Moist and Dry groups were placed in their respective chambers and the two Normal groups were sampled for analysis. The bark was subdivided radially into three zones, and each zone was weighed and analysed separately. The wood was treated in the usual way.

The determinations made on the sap were: total crystalloid N, asparagine N, amino-acid N, sucrose and reducing sugars. Total N was determined as usual on the dried material.

The object of the radial subdivision was to ascertain in what zone of the bark the changes of the protein-crystalloid N ratio occurred, while the determinations of the various crystalloid fractions were undertaken in order to ascertain what crystalloid fractions were involved.

Results.

(1) *Changes in the bark as a whole.* The first consideration is the basis on which the results are to be expressed. The initial fresh weight of each set of quadrants is known. As the combined fresh weight of the four sets of quadrants from each original sample of stems is also known, we can calculate what fraction each set is of the original sample before subdivision. On the assumption that the ratio of bark to wood is the same for all sets, we can also calculate, from the amount of any substance found, per quadrant, in any set, the amount which would be present in the whole stem. For example, the initial fresh weight of one of the sets of quadrants from the A sample was 26.85 per cent. of the sum of the initial fresh weights of all four sets of quadrants from the A sample. If the amount of nitrogen found, per quadrant, in this set is x grm. the amount per stem is $100x/26.85$ grm. The amounts of water, nitrogen and sugar per stem, calculated in this way, are given in Table I. The figures for the initial group are the means of four sets of quadrants (Normal A 1, A 2, B 1, B 2) and for the Moist and Dry groups the means of two sets (Moist A and B, Dry A and B).

It will be seen (Table I) that considerable changes in moisture content have taken place. In the bark the Moist group shows a slight increase on the Initial collection, while the Dry group is about 27 per cent. down. Both groups show a considerable loss of total sugars, the loss in the Dry group being particularly pronounced. In the wood both groups show a loss of water, but the loss is greatest in the Dry group. The loss of sugar is about the same in the two groups.

TABLE I.

Estimated Amounts of Water and Total Sugar (grm.) and Nitrogen (mg.) in Bark and Wood, per Stem.

	Bark.				Wood.			
	Initial Group.	Moist Group.	Dry Group.		Initial Group.	Moist Group.	Dry Group.	
Water . .	43.93	45.39	32.17	(33.07)	64.47	59.39	51.04	(50.62)
Total Sugars	2.357	1.913	1.603	(1.647)	1.329	1.126	1.218	(1.207)
Total N . .	326.1	334.1	312.7	(320.9)	253.5	257.0	263.2	(261.1)

The estimated total N content of bark and of wood is approximately the same in the Initial and Moist groups, but the Dry group shows an apparent loss from the bark and a gain by the wood. The total N in the stem (bark + wood) of this group is 575.9 mg., which is almost identical with that in the Initial group, viz. 579.6 mg. This might be the result of transport of N from bark to wood, but it seems more probable that it is due to the fact that one of the sets of quadrants in the Dry group had rather less bark and more wood than the corresponding sets of quadrants in the Initial and Moist groups. That this is the case seems clear from inspection of the ratios of the dry weights of bark and wood in all sets. In Table II the dry weight of the bark is expressed as a per cent. of the dry weight of bark + wood for each set of quadrants.

TABLE II.

Dry Weight of Bark as a Per Cent. of Dry Weight of Stem (Bark + Wood).

	Initial Group.		Moist Group.	Dry Group.	Mean of all Groups.
	1.	2.			
A sample	23.66	22.91	23.83	23.21	23.40
B „	24.12	23.87	23.73	22.19	23.48

In the A sample there is little difference between Initial, Moist, and Dry groups. In the B sample, however, the Initial and Moist groups are very similar and high, while the Dry group is quite low. If the values for this sample are corrected, on the assumption that the bark values are too low in the ratio 22.19/23.48 and the wood values are too high in the ratio 77.81/76.52, we obtain, for the total N content of the Dry group, Bark 320.9 and Wood 261.1, figures which differ very little from those for the Initial group.¹ It seems unlikely, therefore, that there has been any movement of N from bark to wood, or vice versa, in any group, and the assumption that the total N content in bark and in wood has been unchanged would appear to be justified. The changes in the N fractions can therefore be followed by expressing each as a percentage of the total N present.

¹ The means for the Dry group, corrected in this way, are shown in brackets in Table I.

TABLE III.
Percentage Composition of N in Bark and Wood.

	Bark.			Wood.		
	Initial Group.	Moist Group.	Dry Group.	Initial Group.	Moist Group.	Dry Group.
Protein N. . .	51.39	58.31	66.38	66.54	67.55	65.60
Crystalloid N . .	48.61	41.69	33.62	33.46	32.45	34.40
Asparagine N . .	38.28	33.84	27.43	11.69	12.02	12.58
Amino-acid N . .	3.16	0.48	-0.25	4.69	2.24	2.78
Residual + Nitrate N	7.17	7.37	6.44	17.08	18.19	19.04

(Heavy type figures in Moist group show a significant gain or loss on the Initial.)
 " " " Dry " " " " " " " Moist.)

TABLE IV.
Concentrations in Bark.

(Grm. per 100 grm. water.)

	Initial.	Moist.	Dry.
Total Sugars . . .	5.366	4.217	4.994
Total Crystalloid N . .	0.3602	0.3068	0.3252

It will be seen (Table III) that in both Moist and Dry groups protein has increased at the expense of crystalloid N. That a change in this direction should have occurred, in spite of a reduction in sugar concentration (Table IV), is perhaps not surprising, for the proportion of crystalloid N at the Initial collection (48.61 per cent.) is higher than the average (40.12) for this region of the stem. In the Moist group, where no loss of moisture has taken place, the proportion of crystalloid N is reduced to 41.69, only slightly above the average figure. In the Dry group, however, the proportion of crystalloid N is still further reduced, viz. to 33.62.

There would appear to be two possible explanations of this further effect due to drying. In the first place the concentration of sugar is higher in the Dry than in the Moist group (Table IV). This in itself should tend to make the proportion of crystalloid N lower in the Dry group. Secondly, desiccation might tend, by reducing the concentration of water, to change the equilibrium in the direction of protein. Comparing Moist and Dry groups, it will be noted that the amount of crystalloid N converted to protein as a result of desiccation is a little short of the amount of conversion required to maintain a constant concentration of crystalloid N. The relative drop in proportion of crystalloid N is smaller than the relative drop in

moisture content, so that the *concentration* of crystalloid N actually increases slightly. Thus, conversion of crystalloid N to protein N, as a result of desiccation, would appear to proceed no further than is required to keep the concentration of crystalloid N approximately constant.

In the rapid changes of ratio, referred to earlier (p. 241), the behaviour is quite different. Moisture content falls very slightly, and the reduction in proportion of crystalloid N involves an approximately equivalent reduction in concentration. Thus, in Experiment 3, the mean decreases within a period of three hours were: moisture content 1.8 per cent., proportion of crystalloid N 27.6 per cent., and concentration of crystalloid N 27.5 per cent. of the initial values for each.

It would appear, therefore, that these rapid changes in ratio cannot be attributed to changes in moisture content.

With regard to the crystalloid fractions which take part in conversion of crystalloid N, it will be seen (Table III) that the 'adjustment' effect, which we take to be the difference between the Initial and Moist groups, is due to asparagine and amino-acids, the relative change being greatest in amino-acids. The residual + nitrate fraction remains unchanged. The change due to desiccation (difference between Moist and Dry groups) apparently involves all the crystalloid N fractions. The asparagine loss is the greatest and is *per se* significant. Whether amino-acids and residual N make any real contribution cannot be decided, for the differences are not significant.

A word should now be said concerning the changes that occurred in the wood (Table I). Both Moist and Dry groups show a loss of water, but the loss is, of course, greater in the Dry group. The loss of sugar is about the same in the two groups. It will be seen that the amount of total N, as already noted, shows practically no change. The proportion of crystalloid N (Table III) is initially lower than in the bark and *remains approximately unchanged throughout the experiment*. Amino-acids decrease, as in the bark, though not to so marked an extent. In the bark, however, the drop in amino-acids is due to direct or indirect conversion to protein. In the wood, on the other hand, the gain is shared by asparagine and residual N. To sum up, the stability of crystalloid N in the wood is in contrast to its lability in the bark.

(2) *Changes in inner, middle, and outer zones of bark.* The bark was subdivided into three zones, in order to ascertain in what zone the change in proportion of crystalloid N occurred. Comparison of the groups is rendered fairly simple from the fact that the subdivision into outer, middle, and inner fractions was reasonably uniform, the average fraction of the total fresh weight removed at each stage being very nearly the same in all three groups (Table V). In consequence it seems justifiable to make a direct comparison between the groups for each of the zones.

TABLE V.

Radial Subdivision of Bark.

Average Fresh Weight of each Zone as Fraction of Total Fresh Weight of Bark.

Zone.	Initial Group.	Moist Group.	Dry Group.	Standard Deviation for One Sample.
Outer	0.374	0.355	0.377	0.026
Middle	0.289	0.303	0.282	0.021
Inner	0.337	0.342	0.341	0.019

Although there was a considerable reduction in moisture content in the Dry group, it appears as if this reduction had affected all zones equally. For if we divide the fresh weight/dry weight ratio for each zone by the fresh weight/dry weight ratio for the whole bark, so as to obtain a figure representing the *relative moisture content* of each zone, we find that the relative moisture contents are very nearly the same in all three groups. The mean values for each group are given in Table VI.

TABLE VI.

Relative Moisture Contents of Inner, Middle, and Outer Zones of Bark.

Zone.	Initial Group.	Moist Group.	Dry Group.	Standard Deviation of Difference between means of Two Samples.
Inner	1.056	1.065	1.050	0.013
Middle	1.047	1.040	1.060	0.014
Outer	0.929	0.918	0.926	0.017

Consequently, when a sample of bark has lost x per cent. of its water, as compared with the Initial collection, we can fairly assume that each zone has lost x per cent. of its water. Making this assumption, we can use the *concentrations* of total N found in each zone to test whether there has been any appreciable movement of nitrogen from one zone to another. Expressing the concentration in each zone in terms of the concentration in the bark as a whole, we obtain figures for the relative concentrations in each zone. The mean figures for each group are given in Table VII.

TABLE VII.

Relative Concentrations of Total N in Inner, Middle, and Outer Zones of Bark.

Zone.	Initial Group.	Moist Group.	Dry Group.	Standard Deviation of Difference between means of Two Samples.
Inner	1.098	1.092	1.097	0.021
Middle	1.000	1.003	1.004	0.023
Outer	0.913	0.917	0.910	0.026

It is clear from these figures that there has been no appreciable change in the total N content of any zone: accordingly, the changes in the N fractions can, as in the case of the whole bark, be determined by expressing each as a per cent. of the total N present. The changes observed are shown in Table VIII, which gives the values for the Initial group and the loss or gain by the Moist and Dry groups. The figures recorded are the means of the values for the individual samples, and statistically significant losses or gains are in heavy type.

TABLE VIII.

Percentage Composition of Total N in Inner, Middle, and Outer Zones of Bark.

	Bark.								
	Initial Group.			Loss or Gain of Moist Group on Initial Group.			Loss or Gain of Dry Group on Moist Group.		
	Inner.	Middle.	Outer.	Inner.	Middle.	Outer.	Inner.	Middle.	Outer.
Crystalloid N	54.05	54.53	37.67	-9.28	-8.13	-4.35	-8.32	-12.76	-2.78
Asparagine N	38.99	45.23	31.59	-5.48	-5.66	-3.12	-6.49	-10.62	-1.72
Amino-acid N	4.63	2.68	2.01	-3.28	-2.45	-2.35	-0.61	-1.06	-0.57
Residual N + Nitrate N	10.43	6.62	4.07	-0.52	-0.02	+1.12	-1.22	-1.08	-0.49

We will consider first the change in the Moist group, which we interpreted as an adjustment to the average conditions of protein-crystalloid N equilibrium. It will be seen that the diminution in crystalloid N took place mainly in the Inner and Middle zones and was due largely to asparagine. The remainder of the decrease is accounted for by amino-acids, which appear to have disappeared entirely from the Outer zone. The residual + nitrate fraction shows hardly any change. This conversion of crystalloid N to protein appears, therefore, to be mainly a conversion of asparagine and amino-acids in the Inner and Middle zones of the bark.

The further decrease in crystalloid N due to desiccation is also found mainly in the Inner and Middle zones of the bark. Owing, however, to the fact that the amino-acids are already considerably reduced in the Moist group, this fraction scarcely shares in the decrease. The bulk of the decrease is in asparagine, but there is also a small change in the residual + nitrate fraction.

To sum up, the change in the crystalloid-protein N ratio takes place mainly in the Inner and Middle zones of the bark, and involves principally asparagine and amino-acids. When the change is due to desiccation, the residual + nitrate fraction may also be involved, though only to a small extent.

(d) Summary on Changes of Ratio in the Bark.

A graphical summary of the observed changes in proportion of crystalloid N in the whole bark is presented in Fig. 1. The proportion of

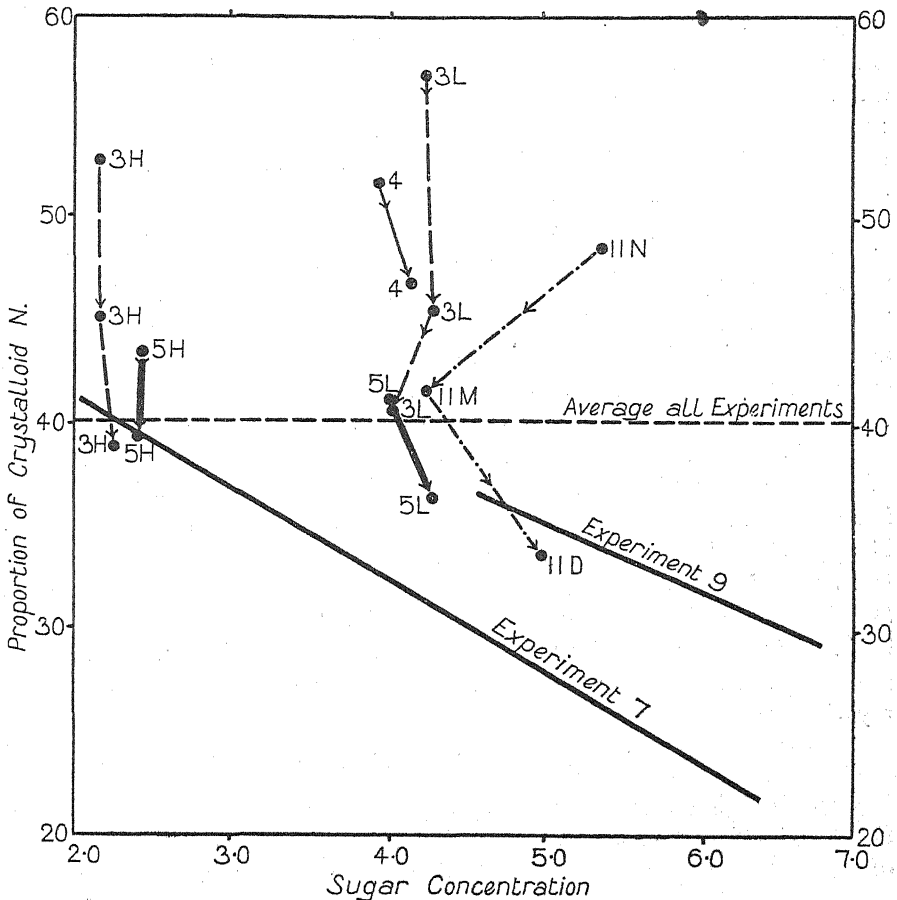


FIG. 1. Graphical summary of fluctuations in crystalloid-protein N ratio. Crystalloid N as a per cent. of total N is plotted against total sugar concentration. 1. The horizontal line shows the average value (lower region of stem) for all experiments. 2. The sloping lines show the regression of proportion of crystalloid N on total sugar concentrations in Experiments 7 and 9. 3. Rapid changes of ratio in Experiments 3, 4, and 5 are shown by the numbered points connected with arrows which show the direction of change in time. The subscripts H and L after the experiment numbers indicate that there was a ring above (H) or below (L) the region of stem sampled. Note the parallel change of ratio in High-ring and Low-ring groups at widely different sugar concentrations. 4. 11 N is the initial value of Experiment 11, 11 M is the moist group and 11 D the dry group. The arrows show the time adjustment of the ratio, 11 N to 11 M, and the further change due to desiccation, 11 M to 11 D.

crystalloid N is plotted against sugar concentration. The sloping lines, marked Experiment 7 and Experiment 9, show the regression of the proportion of crystalloid N on total sugar concentration for these two experiments.

The horizontal line represents the average proportion of crystalloid N in the Lower region for all experiments. Experiments (Nos. 3, 4 and 5) in which rapid changes of ratio took place are shown by the points connected with arrows. The arrows show the direction of change in time. The subscripts H and L after the experiment numbers indicate that there was a ring above (H) or below (L) the region of stem sampled. It will be noted that the ringing experiments 3 and 5 show parallel changes in ratio at widely different sugar concentrations. 11 N is the initial value for Experiment 11, 11 M represents the Moist group and 11 D the Dry group. The arrows show the adjustment effect in time (11 N to 11 M) and the further change due to desiccation (11 M to 11 D).

From the data presented in the preceding section, the following tentative conclusions may be drawn: (1) The proportion of crystalloid N in the bark tends to increase as the concentration of sugars diminishes. The effect is shown in both inner and outer halves of the bark, but the changes in ratio are more marked in the inner half, owing to the fact that the change in sugar concentration is greater there. The effect of change in hydrogen ion concentration is uncertain; (2) Desiccation may cause a decrease in the proportion of crystalloid N but not a decrease in concentration; (3) A rapid fall in proportion of crystalloid N is generally found when the initial value is much above the average; (4) The conversion of protein N to crystalloid N appears to involve mainly asparagine and amino-acids, the remaining fraction (residual + nitrate N) not being affected to any appreciable extent; (5) The change is largely confined to the inner and middle zones of the bark, which consist mainly of sieve-tubes and ray-tissues. It appears probable that the change takes place in the sieve-tubes as well as in the ray-tissues.

(e) *Changes of Ratio in Wood and Leaf.*

In discussing Experiment 11, reference was made to the stability of the protein of the wood. Other experiments further emphasize this stability of the protein in the wood as compared to that in the bark. Thus in those experiments (Nos. 3, 4 and 5), in which the proportion of crystalloid N in the bark showed a rapid decrease in the course of the day, the proportion in the wood showed no appreciable change. The initial value in the wood in these cases was, however, not far removed from the average value, and little change would, perhaps, be expected. On the other hand, in Experiment 7, in which a leafy stem was compared with one defoliated some five days earlier, the change in the wood was in the same direction as that in the bark, i. e. there was an increase in the proportion of crystalloid N in the leafless stem and a parallel decrease in sugars. The data for the wood are not, however, sufficiently complete for any test of the association between the proportion of crystalloid N and sugar concentration. It would appear

then that while a severe reduction in sugar concentration may lead to the same changes in the wood as in the bark, the ratio in the wood is not appreciably affected by those factors which cause rapid fluctuations in the bark, independently of any change in sugar concentration. Also desiccation does not, in the wood, cause a conversion of crystalloid N to protein N, as it does in the bark. It is of interest also to note that the wood, unlike the bark, shows no consistent difference between the Upper and Lower regions of the stem.

In the *leaf* the proportion of crystalloid N varies very little either within an experiment or from one experiment to another; the extreme range is from 9.0 to 12.86, though the sugar concentration ranges from 0.5 per cent. to 3.25 per cent. There does not appear to be any relation between sugar concentrations in different experiments and the proportion of crystalloid N. Such general changes as do occur in any experiment can be referred to the balance between synthesis and translocation. Thus, in a diurnal experiment, which will be described in a later paper, the proportion of crystalloid N was significantly higher at evening than at dawn, 12.57 as against 11.68. In an experiment described in the present paper (Experiment 2) the diurnal change was in the same direction, but was not statistically significant. That the increase in the proportion of crystalloid N in the leaf by day and decrease by night is a general phenomenon is suggested by the fact that in the Runner bean Chibnall (1) found the same relation.

(f) *Discussion.*

The conditions determining the synthesis and degradation of protein in the plant have been studied by a large number of workers. Most of the work has been carried out, however, on seedlings, leaves, developing fruits, or whole stems. Conditions in the conducting tissues have not apparently been studied hitherto. Nor do such rapid 'adjustment' changes as we find in the bark appear to have been previously observed. It is a little difficult, therefore, to compare our results with those of other workers.

The association which we find between sugar concentration and protein synthesis or degradation is in harmony with the conclusions of Prianischnikow (8), Monteverde (6), and other workers. As regards the crystalloid N compounds which take part in this type of conversion, the workers cited agree in assigning chief importance to asparagine and its regeneration product ammonia. In the experiment which showed this type of conversion most clearly (Experiment 7), we did not analyse the crystalloid N and cannot, therefore, say what fractions were involved.

In the case, however, of the rapid changes in ratio in the bark, which are independent of sugar concentration, it is clear that asparagine and amino-acids are the principal compounds involved. This observation lends

force to the suggestion of Woodman and Engledow (9) that there may be a direct condensation of asparagine and amino-acids to form protein instead of a more complex chemical reaction involving carbohydrates.

As to the effect of desiccation on the protein-crystalloid N ratio we are not aware of any previous observations on the bark. Mothes (7) found for *leaves* that crystalloid N increased as the moisture content decreased. In the bark we found the reverse effect, while in the wood we could detect no change.

From the point of view of nitrogen transport the most important result of this survey of the protein-crystalloid N relations is the great lability of protein in the bark, especially the inner part. The concentration of crystalloid N in the bark of any region is thus only in part determined by the balance between import and export, and may exhibit very marked fluctuations due to its conversion into protein N or vice versa. The two tentative generalizations that we have been able to make, namely, that a conversion of crystalloid N into protein N is probable (*a*) when sugar concentration increases, and (*b*) when the proportion of crystalloid N is, initially, very much above the average, may, however, be of some use in interpreting the results of ringing experiments.

As to the significance for the general problem of transport of these observations on lability, it will be evident that if all the crystalloid fractions, but none of the protein, are mobile in the sieve-tube, such rapid changes in the proportion of crystalloid N as those observed, for example, in Experiment 3, might cause violent fluctuations in the rate of transport from the leaf via the bark to other parts of the plant. For neither leaf nor wood appear to partake of these rapid changes. If protein also is mobile in the sieve-tube, movement longitudinally in the bark might not, in itself, be much affected. But although we can, perhaps, conceive of protein moving from one sieve-tube to another, we can less readily imagine the movement of a colloid across parenchyma cells to and from the sieve-tubes. Thus while an increased concentration of protein might partly compensate for a decreased concentration of crystalloid N, so far as movement along the sieve-tube is concerned, movement into and out of the sieve-tube would presumably be determined solely by the concentration of crystalloid N. Transport from the leaves via the bark to the rest of the plant might in consequence be violently disturbed by these rapid changes in proportion of crystalloid N.

If, however, the form in which crystalloid N enters or leaves the sieve-tubes is a compound which does not share in these rapid changes, such violent disturbances in rate of movement would not occur. It is interesting in this connexion to note that the 'adjustment' effect in Experiment 11 was due to the conversion of asparagine and amino-acids, while the residual N + nitrate N fraction showed little change. In Experiment 3, again,

asparagine was responsible for the greater part of the conversion (see later, p. 259); the remainder was due to amino-acids and residual N, which were not estimated separately. Arguing from the results of Experiment 11, this change in the amino-acid + residual N fraction might well be due to amino-acids alone. If it could be definitely established that residual N does not take part in these changes, and that the changes do not affect the rate of transport of nitrogen about the plant, there would be grounds for identifying residual N as the mobile form, at least for movement into and out of the sieve-tube. Within the sieve-tube all fractions might still share in the movement. The data available are not, however, sufficient to decide these questions.

SECTION 3. THE EFFECTS OF RINGING.

(a) *Experiments with Undivided Bark (Experiments 2, 3 and 6).*

Experiment 2.

This experiment was carried out primarily in order to determine whether there was a negative gradient of crystalloid N in the bark when downward movement of nitrogen was proceeding. The gradients found in the normal plants and the evidence of downward movement have already been presented (3). Attention will therefore be confined here to the response of protein and crystalloid N to ringing and to diurnal changes in the rate of transport out of the leaf.

Procedure. The experiment was carried out on October 6 and 7, 1927. The plants were thirteen weeks old and were without flowers or bolls. There were two groups: the plants of the Normal group were marked with tape just below the first fruiting-branch, and from those of the Ringed group a ring of bark was removed at the same level. Two regions of stem were taken for analysis; the Upper region consisted of the 20 cm. section of stem immediately above the ring or tape and the Lower of the similar section immediately below the ring or tape. In the laboratory all the stem samples were trimmed to 18 cm., the portions abutting on the first fruiting-branch being rejected.

An initial collection of Normal plants was made at the same time as the operation of ringing viz., 5.30 a.m. Subsequent collections of Normal and Ringed plants were taken at 11.0 a.m. and 4.30 p.m., and also at 5.30 a.m. on the following morning. For each collection there were two samples from each group. Each sample consisted of forty plants. One leaf was taken also from the main axis of each plant in the region between the seventh and tenth nodes from the apex. The leaves were graded by length of lamina. Determinations were made of total N, crystalloid N, total sugars and polysaccharides.

Results. The results for carbohydrates were of the usual type (5) and

are not described in detail. There were diurnal changes in absolute amount as well as in concentration of sugars in leaf and bark. Sugars also accumulated in leaves, bark, and wood above the ring, and decreased in bark and wood below the ring.

The diurnal change in total N content of the normal plants is summarized in Table IX, where the mean of the two collections at dawn (5.30 a.m. on October 6 and 7) is compared with the mean of the two collections during the day (11.0 a.m. and 4.30 p.m. on October 6). The values given are calculated on the basis of the mean residual dry weight,¹ since the experiment was of short duration and the residual dry weight showed no significant change in time, or as between Normal and Ringed groups. On the right of the table is given the level of significance, calculated from the sampling variation, by which the observed *excess* of the day over the dawn values may be tested. It will be seen that in all cases there is an excess of the day over the dawn values, and that this excess is statistically significant in the case of the leaves and the Upper region of bark. In the Lower region of bark the observed diurnal change is too small to be judged significant.

TABLE IX.

Mg. Total N per Leaf and per 18 cm. of Bark (Normal Group).

	Dawn Collections.	Day Collections.	Excess of Day over Dawn.	Significant Excess (P = 0.05).
Leaves	106.41	110.43	4.02	3.73
Bark Upper Region	36.16	38.84	2.68	2.43
Bark Lower Region	55.51	57.80	2.29	3.80

In all cases the greater part of the diurnal change appeared to be due to the protein N fraction; when tested statistically, however, in neither the crystalloid nor the protein fraction was the individual change significant.

For the measurement of the response to ringing we compare the mean for the three Normal collections at 11.0 a.m., 4.30 p.m., and 5.30 a.m., with the mean for the Ringed collections made at the same times. The results are given in Table X. As before, the values are calculated on the basis of the mean residual dry weight. In the leaves and Upper region of bark we expect (cf. 2) an excess of the Ringed over the Normal group, and in the Lower region of bark an excess of the Normal over the Ringed. The excess in the specified direction which might have occurred merely by chance in 5 per cent. of cases is given on the right of the table.

The accumulation of total N in the leaves of the Ringed plants is definitely established:² the greater part of the accumulation is due to protein N, although the percentage change is greater for crystalloid N.

¹ Residual dry weight = Dry weight less total carbohydrates (cf. 5).

² In Part I (2) a small increase was noted, but there the increase was not statistically significant.

The changes in crystalloid N and protein N are not, however, individually significant, and no satisfactory distinction between the crystalloid N and the protein response can be drawn. In the Upper region of bark there is a significant response of crystalloid N as well as of total N, while the change in protein N is very small. In the Lower region, on the other hand, it is the protein fraction which shows a significant response, the change in crystalloid N being relatively small.

TABLE X.

Mg. Total N per Leaf or per 18 cm. of Bark.

	Mean Excess of Ringed over Normal.			Significant Excess ($P = 0.05$).		
	Total N.	Protein N.	Crystalloid N.	Total N.	Protein N.	Crystalloid N.
Leaves	3.905	3.137	0.768	3.048	3.705	1.655
Bark Upper Region	3.890	0.395	3.495	1.983	1.655	1.183
Bark Lower Region	-4.015	-2.870	-1.145	3.120	2.380	1.618

To appreciate the possible transport significance of the changes observed, we will assume for the moment that transport occurs mainly as some form of crystalloid N. On this view there should be, initially, in the Upper region of the bark of the Ringed group an accumulation of crystalloid N. This would disturb the protein-crystalloid N equilibrium, and part of the crystalloid N would be converted to protein N. At the same time the sugar concentration would be increasing, and this, as we have seen, would further tend to diminish the proportion of crystalloid N. Consequently, after some time, the accumulation of nitrogen above the ring might appear as an accumulation mainly of protein N. How long the initial phase (i.e. the accumulation of crystalloid N) lasted would depend on the relative speeds of arrival of crystalloid N and of conversion to protein N. Below the ring the initial phase should be a drop in crystalloid N; protein N should then be converted to crystalloid N, to restore the equilibrium between protein N and crystalloid N, and there should be a further conversion of protein N to crystalloid N as a result of the marked fall in sugar concentration. Thus, both above and below the ring, one might expect, initially, a response mainly of crystalloid N, followed by a response mainly of protein N.

In the present case the changes between individual collections are of roughly the same order as the standard deviation due to sampling, and only the average effect over the whole time can be measured with any accuracy. Thus, although the drift of the protein-crystalloid N ratio in time did in fact appear to follow the course outlined above, no significance can be attached to this fact. It may be noted (Table XI) that the decrease

of sugar concentration (below the normal value) in the Ringed group under the ring is greater than the increase of sugar concentration in that group above the ring. Conversion of crystalloid N to protein N above the ring might, on this account, be less than the conversion of protein N to crystalloid N below the ring. While this is a possible explanation of the fact that there appears to be a crystalloid N response above and a protein response below the ring, it is only a very tentative one.

TABLE XI.

Average Concentration of Total Sugars in Ringed and Normal Groups.

	Leaves.	Bark Upper Region.	Bark Lower Region.
Normal . . .	1.085	5.811	4.609
Ringed . . .	1.808	6.919	2.809

It would appear, therefore, that the results can be harmonized with the view that movement of nitrogen occurs mainly as some form of crystalloid N, but the possibility that both protein N and crystalloid N are mobile is not excluded. On the other hand, the fact that in the Upper region of bark we have a crystalloid response, in spite of a parallel increase of sugar concentration, seems to exclude the possibility that nitrogen compounds move as protein only.

The changes in the wood are of less importance from the point of view of transport. It is interesting, however, to note that in both the Upper and Lower regions there was a significant response in crystalloid N, but very little change in protein N.

Experiment 3.

In this experiment all the crystalloid N fractions, with the exception of amino-acids, were determined. No attempt was made to follow the time sequence of changes following ringing, but, by taking five samples on the next day, it was hoped to obtain a reliable measure of the response made by the crystalloid N fractions. Instead of determining the changes in the Upper and Lower regions of bark, i. e. both above and below the ring on a stem, only one region of stem was used. In the High-ring group a ring of bark was removed immediately above this region of stem, while in the Low-ring group a ring was removed immediately below the specified region. In the Low-ring group nitrogen accumulates, and in the High-ring group it diminishes. The total response to ringing is measured by the difference between the Low-ring and High-ring groups.

Procedure. The plants were sixteen and a half weeks old, and were without flowers or bolls. They were graded on the basis of circumference measurements in the region of the stem to be sampled. There were five

samples, each of forty-eight plants, for each group. The plants of the High-ring group were ringed just below the first fruiting branch, and were marked with tape 20 cm. below the ring. The plants of the Low-ring group were marked with tape just below the first fruiting branch, and were ringed 20 cm. below the tape. When samples were collected, the stem was cut at the tape and the ring. In the laboratory the samples were trimmed to a length of 18 cm., measured from the lower end, and weighed. The time-table of operations was as follows:

October 31, 7.0 a.m.-9.0 a.m. Ringing.

November 1, 12.30 p.m. Collection of 1st and 2nd samples from each group.

„ 2.15 p.m. Collection of 3rd sample from each group.

„ 3.30 p.m. Collection of 4th and 5th samples from each group.

The determinations made on the sap were: total crystalloid N, asparagine N, ammonia N, nitrate N, and total sugars. Total N and polysaccharides were determined on the dried material.

Results. There was no initial collection, so that it is uncertain whether there was any change in the residual dry weight in time. The dry weights are quite different for the two groups, but the residual dry weights, both for bark and for wood, are very close. Though the values for the Low-ring group are slightly greater, the difference is not significant. The results are, therefore, calculated on the basis of the mean residual dry weight. The effect of this may be to diminish slightly the real difference between the two groups. The residual dry weights are given below:

Mean Residual Dry Weights per Stem.

	Low-ring Group.	High-ring Group.	Significant Difference ($P = 0.05$).
Bark . . .	6.050	6.004	0.208
Wood . . .	20.035	19.491	0.637

The results for the bark, expressed as mg. nitrogen or grm. carbohydrate for a mean residual dry weight of 6.027 grm., are shown in Table XII.

It will be seen that there is a significant difference in sugars, polysaccharides and total N in favour of the Low-ring group. The nitrogen response is due almost entirely to crystalloid N, and the crystalloid N response is *per se* significant, while the small change in protein N is not. Asparagine shows a significant response, which is more than enough to account for the response in crystalloid N. Ammonia shows a positive response which is not, however, significant, while the residual + amino fraction shows a small negative response, which again is not significant. Nitrate N is quite definitely lower in the Low-ring group. Thus the accumulation

of nitrogen, on ringing, in this experiment is almost entirely an accumulation of asparagine.

TABLE XII.

Grm. Carbohydrate and Mg. Nitrogen per Stem in Bark.

	Bark.		Significant Difference ($P = 0.05$)
	Low-ring Group.	High-ring Group.	
Total Sugars . . .	1.322	0.718	0.087
Polysaccharides . . .	1.524	1.172	0.104
Total N . . .	210.7	196.3	5.48
Protein N . . .	109.1	106.5	9.60
Crystalloid N . . .	101.6	89.8	9.85
Asparagine N . . .	70.04	56.25	9.06
Ammonia N . . .	0.85	0.57	0.35
Nitrate N . . .	3.93	4.88	0.53
Amino-acid N + } Residual N }	26.79	28.12	7.79

In view of the fact that the average concentration of sugars in the Low-ring group is very much greater than that in the High-ring group (4.148 grm. as against 2.211 grm. per 100 grm. water), it might have been expected that the proportion of crystalloid N would have diminished in the Low-ring group, and that the accumulated nitrogen would appear as protein N. This experiment, however, is an example of the type noted previously (p. 241), in which we have, initially, an abnormally high proportion of crystalloid N, and during the day a rapid adjustment to the mean value. This fall runs closely parallel in the two groups, so that the average values for the two are sufficient to characterize the change in time. The data are given in Table XIII.

TABLE XIII.

Composition of Total N in the Bark as a Percentage of Total N.

A. Mean of High- and Low-Ring Groups.

Collections.	Hours.	Hours from Ringing.	Total Crystal- loid N.	Aspara- gine N.	Ammonia N.	Nitrate N.	Amino- Acid + Residual N.
1 and 2	12.30 p.m.	28.5	54.89	36.64	0.287	2.236	15.73
3	2.15 p.m.	30.25	45.38	30.33	0.379	2.094	12.58
4 and 5	3.30 p.m.	31.50	39.77	25.48	0.396	2.155	11.74
Significant Difference between means of 2 Collections		$P = 0.05$ $P = 0.10$	4.81	3.95	0.204	0.290	4.72 3.70

B. Mean of the 5 Collections for each Group.

Low-ring Group	48.12	33.18	0.405	1.865	12.67
High-ring Group	45.75	28.65	0.293	2.485	14.32
Significant Difference between Groups	4.30	3.53	0.182	0.259	4.23

The very rapid fall in the proportion of crystalloid N during the afternoon (Table XIII, A) is principally a fall in asparagine, but partly also in the amino-acid + residual N fraction. The change in the proportion of the latter fraction between the first two and the last two collections is not fully significant (P lies between 0.10 and 0.05).

Comparing the High- and Low-ring groups (Table XIII, B), we note a significant increase in the proportion of asparagine in the Low-ring group, and a significant decrease in the proportion of nitrate N; the other differences are not significant. It will be seen that in spite of the higher sugar concentration in the Low-ring group the average proportion of crystalloid N is somewhat higher in that group.

As already pointed out, the meaning of the very high initial proportion of crystalloid N in both groups is not clear, but the sudden fall suggests a rapid realization of equilibrium, for the final value, 39.77, is not far from the average value, 40.12, for this (Lower) region of bark. It is of interest also to find again that the lability of the crystalloid N is largely due to the asparagine fraction. The transport significance of this lability has already been discussed.

Changes in the wood. The results for the wood, which are expressed on the basis of the mean residual dry weight, are given in Table XIV.

TABLE XIV.

Grm. Carbohydrate and Mg. Nitrogen per Stem in Wood.

	Low-ring Group.	High-ring Group.	Difference High—Low.	Significant Difference ($P = 0.05$)
Total Sugars . . .	0.671	0.345	0.326	0.091
Polysaccharides . .	4.108	3.862	0.246	0.097
Total N . . .	219.5	191.6	27.9	13.77
Protein N . . .	123.0	110.2	12.8	10.37
Total Crystalloid N .	96.48	81.41	15.07	14.50

The carbohydrate results are very similar to those recorded for the bark, the greater part of the response being due to sugars. The polysaccharide response is, however, fully significant. The difference in total N is about equally due to the protein and crystalloid N fractions; in both, the individual difference is significant. It will be noted that the percentage change is much greater for crystalloid N, so that the Low-ring group has a slightly higher proportion of crystalloid N, on the average 43.96, as against 42.53; this difference is not, however, statistically significant. As already emphasized, there was in the wood no indication of that rapid fall in the proportion of crystalloid N which is the outstanding feature of the results for the bark. The average proportion of crystalloid N was: Collections 1 and 2, 42.8; Collection 3, 46.3; Collections 4 and 5, 42.2.

Experiment 6.

In this experiment the data are not so complete as in the last, but the phenomenon of rapid change in proportion of crystalloid N in the bark during the day is absent, and interpretation is therefore somewhat simpler.

The region of stem sampled was, as in the last experiment, immediately below the first fruiting branch. Two groups of plants, a Low-ring and a Two-ring, were compared. Both groups had a ring 18 cm. below the first fruiting branch. One of them, the Two-ring group, had also a ring immediately below the first fruiting branch. The stems were trimmed to 15 cm. from the lower ring and weighed. Ringing took place at 6.45 a.m. on December 5, and five samples from each group were collected during the following day, at 9.15 a.m., 10.25 a.m., 12.40 p.m., 1.40 p.m., and 3.30 p.m. Each sample consisted of forty-nine plants. Total sugars, total crystalloid N, and asparagine N were determined on the sap, and polysaccharides and total N on the dried material. No collection was made at the time of ringing. Crystalloid N was not determined in the wood.

Results. The samples from the Low-ring group had a somewhat higher dry weight, but the mean residual dry weights (per stem) for the two groups are almost identical, viz. Bark: Low-ring, 2.691 grm., Two-ring, 2.689 grm. Wood: Low-ring, 6.683 grm., Two-ring, 6.575 grm. The results are accordingly expressed on the basis of the mean residual dry weight (per stem) for all samples. Table XV gives the mean values for the five samples of each group.

TABLE XV.

Mg. Nitrogen and grm. Carbohydrate per Stem in Bark and Wood.

	Bark.				Wood.			
	Low-ring.	Two-Ring.	Low Minus Two.	Sig. Diff. (P=0.05).	Low-ring.	Two-ring.	Low Minus Two.	Sig. Diff. (P=0.05).
Total Sugars..	0.673	0.460	0.213	0.068	0.264	0.183	0.081	0.029
Polysaccharides	0.675	0.520	0.155	0.040	1.255	1.186	0.069	0.053
Total N.	82.71	73.71	9.00	4.43	68.41	60.16	8.25	4.27
Protein N.	51.22	43.87	7.35	3.39	—	—	—	—
Total Crystalloid N	31.49	29.84	1.65	2.47	—	—	—	—
Asparagine N	23.06	20.00	3.06	2.20	—	—	—	—
Amino-acid + Nitrate N + Residual N	8.43	9.84	-1.41	0.49	—	—	—	—

The carbohydrate response is of the usual type, and does not call for comment. The nitrogen content of both bark and wood in the Low-ring group is significantly greater than in the Two-ring group. It will be seen that, in the bark, the greater part of the difference in nitrogen is due to protein N, and the difference is fully significant. There is a small response

in crystalloid N, but it is not significant. In asparagine there is a significant increase, but it is offset by a significant drop in the non-asparagine crystalloid N fractions.

The average proportion of crystalloid N is slightly lower in the Low-ring group, 39.08 as against 40.47 in the Two-ring. This small difference between the two groups is statistically significant and may, perhaps, be associated with the higher sugar concentration in the Low-ring group, 4.95 per cent. as against 3.26 per cent. The mean figure for the two groups is just about the average for this region of stem, and the variation during the day was very slight.

(b) *Experiments with Subdivided Bark (Experiment 5).*

Experiment 5.

In this experiment we attempted to determine, by the method of radial subdivision of the bark, in which zone the response to ringing takes place. The ringing procedure followed that already described for Experiment 3, i.e. only one region of stem was sampled. The Low-ring group was ringed just below, and the other group, the High-ring group, just above this region of stem. The region employed consisted of 18 cm. of bare stem below the first fruiting branch. In the laboratory the stems were trimmed to a length of 15 cm.

Ringing took place at 7 a.m. on November 23. On the following day four collections were made from each group, viz. at 9.20 a.m., 10.50 a.m., 1 p.m., and 2 p.m. Each sample taken consisted of fifty-one stems. The bark was subdivided into outer, middle, and inner zones in the manner previously described (5). The wood was not analysed.

Total crystalloid N, asparagine N, ammonia N, sucrose and reducing sugars were determined on the sap, and total N and polysaccharides on the dried material. For the two morning collections the asparagine determinations were unsatisfactory, and had to be discarded. The results for the two afternoon collections showed no significant difference in asparagine content between the Low-ring and the High-ring groups. While, therefore, the asparagine results obtained were of great interest from the point of view of radial distribution, the absence of any significant response to ringing renders it unnecessary to discuss them here. The facts relating to radial distribution of asparagine have been already presented (cf. 3).

Results. In discussing the response to ringing in different zones of bark, it is simplest to work with concentrations. If there has been no change in moisture content this is equivalent to comparing actual amounts. In this experiment there was a small difference between the two groups, for the average weight of water *per sample of bark* was somewhat greater in the Low- than in the High-ring group, the average increase being 2.48 per cent. Expressed on the basis of the *mean residual dry weight* for

all samples, the average increase was only 1.67 per cent. Although in both cases the increases are statistically significant, they are relatively small. If we knew that the increase in water was uniformly distributed in the bark, we might adjust the observed concentrations so as to allow for the increase. In the present experiment, however, the increase appears to be somewhat greater in the middle zone of the bark than in the outer or inner zones, and adjustment of the observed concentrations is not advisable, as the calculations would involve a number of somewhat doubtful assumptions. Accordingly we give the actual concentrations observed (Fig. 2). The effect of presenting the results as concentrations will be to minimize, especially in the middle zone, the difference between the two groups.

As in the previous graphs of radial distribution in the bark, the base line represents the total fresh weight of a sample of bark, and is subdivided in the ratio of the fresh weights of each fraction, outer, middle, and inner, working from left to right. The observed concentration in any zone is plotted above the midpoint of that zone on the base line. As there was some change during the day in sugar concentration, and in the proportion of crystalloid N, the results are shown in two parts. On the left are given the results for the two morning collections, and on the right those for the two afternoon collections. There was no appreciable change during the day in water content or in total N content of the whole bark. The Low-ring group is shown by black circles and continuous lines and the High-ring group by open circles and broken lines.

The results for sugars are very clear-cut and of the usual type. There is a small response of reducing sugars in all three zones, but the major response is that of sucrose in the inner and middle zones. It will be noted that the increase in sugar concentration during the day is principally an increase of sucrose in the inner zone.

On the other hand the response in total N is, at the morning collections, very nearly the same in all three zones. In the afternoon the response appears much greater in the inner zone than elsewhere. If account is taken of the fact that the increase in moisture content in the Low-ring group was rather greater in the middle zone than in the inner or outer zones, it would appear that the response to ringing is about equally marked in inner and middle zones, and somewhat less in the outer zone.

It will be seen that the greater part of the total N response is due to protein N. The greater response of protein N in the inner zone in the afternoon is similar to that shown by total N.

The crystalloid N response is very small, and is apparently absent from the middle zone. This may be due to the somewhat greater moisture content of the middle zone of the Low-ring group.

The important feature in the nitrogen results is that the response is almost wholly due to protein N, and does not appear to be localized in

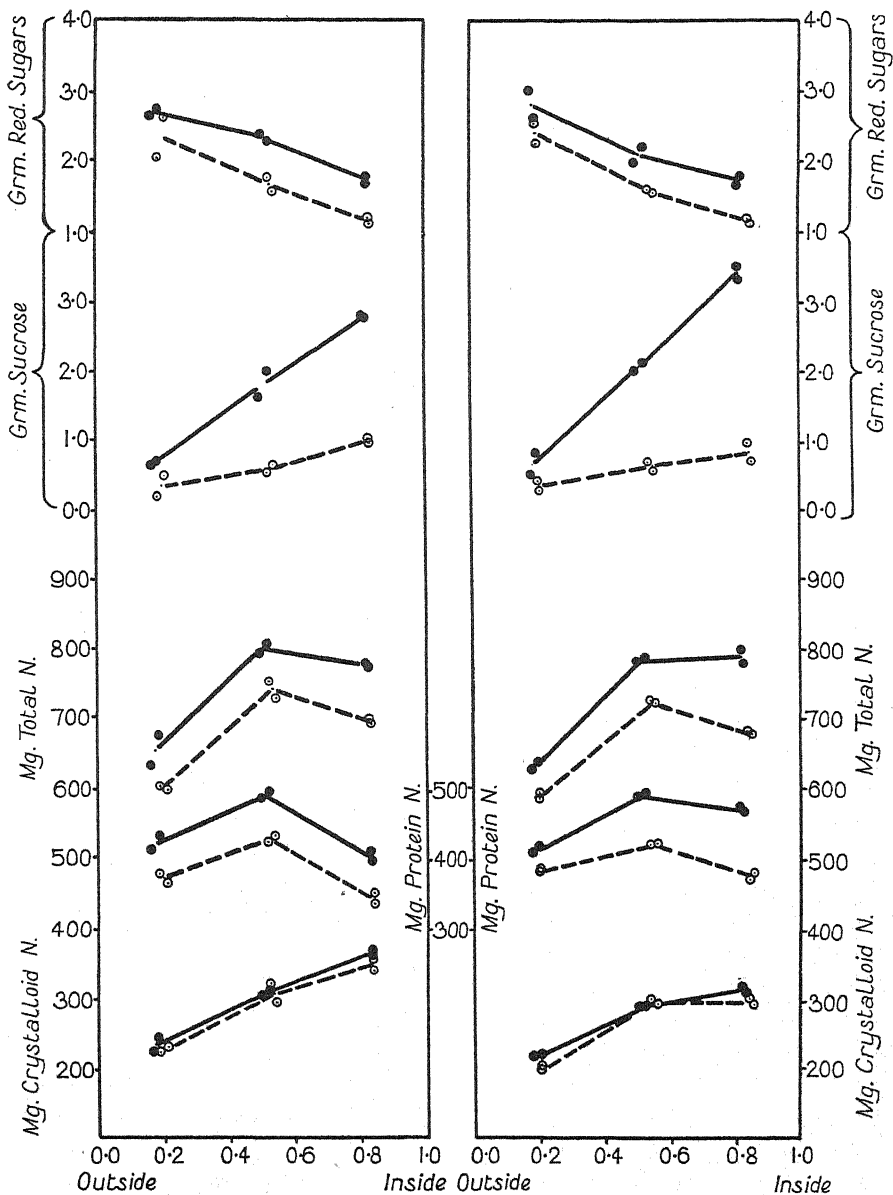


FIG. 2. Radial distribution of sugars and nitrogen in bark. Concentrations (per 100 grm. water) are plotted against distance from outside of bark, the whole distance being unity. On left are shown morning observations and, right, afternoon observations. Continuous line represents Low-ring group and broken line High-ring group.

the inner and middle zones of the bark, in the way that the sugar response is localized.

The proportion of crystalloid N, in the bark as a whole, decreases

during the day, the decrease being rather greater in the Low-ring group. It will be seen from the graphs that this is due almost entirely to an increase of protein N at the expense of crystalloid N in the inner region of the bark. This observation confirms the conclusion reached earlier, that lability of crystalloid N is especially marked towards the inside of the bark.

(c) *Discussion.*

In two of the four ringing experiments reported (Nos. 3 and 5) there were rapid adjustment changes in the proportion of crystalloid N. These changes in the ratio were similar in the Low-ring group, where sugar had accumulated, and in the High-ring group, where the concentration of sugar had diminished. In both experiments the proportion of crystalloid N was not observed at the time of ringing, and the first observations were not made until the following day. In Experiment 3 the response was mainly crystalloid N, and in Experiment 5 mainly protein N. Reference to Fig. 1 may help to explain the meaning of this difference in response. Assuming that, at the time of ringing, the proportion of crystalloid N in both experiments was somewhere about the average value, which is shown for this region of the stem by the horizontal line on the graph, then, in both experiments, for some reason at present unknown, the proportion of crystalloid N increased above the normal value. We may suppose it to have risen in Experiment 3 to somewhere about sixty, and in Experiment 5 to about forty-five. The mean level for the proportion of crystalloid N between the time of ringing and the time when the observations were made would then be much higher in Experiment 3 than in Experiment 5; the response should therefore be mainly crystalloid N in the former and protein in the latter. In general, therefore, where there are rapid adjustment changes in progress, the type of response observed during the interval will depend on the mean level in the proportion of crystalloid N during that interval. As far as our observations show, these rapid changes are not affected by the type of treatment, and are independent of sugar concentration.

In the other two experiments recorded, these rapid adjustment changes were absent, and the type of response observed can be interpreted in terms of sugar concentration. When the observations were made soon after ringing, as in Experiment 2, the accumulation above the ring was mainly crystalloid N, and when, as in Experiment 6, they were made after a lapse of a day, the response was mainly protein. If the main mobile form of nitrogen is crystalloid, the sequence of changes following ringing may be interpreted as the approach to a protein-crystalloid N equilibrium level, determined by sugar concentration. Thus the type of response found in the absence of rapid adjustment changes is in harmony with the view that crystalloid N is the translocatory form of nitrogen, and that the extent to

which it is converted to protein depends on the sugar concentration, while the type of response encountered when rapid changes are in progress, is consistent with either crystalloid N or protein being the translocatory form.

As regards the response of the crystalloid N fractions, the data are incomplete, but it will be noted that, in two out of the three cases where it was determined, asparagine showed a significant response. In each of these cases the asparagine response exceeded the response in total crystalloid N. Whether this indicates that asparagine is largely responsible for longitudinal transport, or whether it is the merely first stage in the sequence from mobile crystalloid N to storage protein cannot be decided on the data; either interpretation will fit the facts.

Much of the difficulty in interpreting the results of ringing experiments would appear to be due to the fact that the rate of longitudinal transport is slow in comparison with the rate at which nitrogen compounds are converted one into another, and with the rate at which nitrogen spreads radially from the sieve-tubes into the other tissues, and vice versa. The results obtained are, in consequence, less well-defined than those obtained for sugars. The great lability of the nitrogen fractions in the bark, especially in the inner zone, suggests that all fractions may share in longitudinal movement within the sieve-tube. Movement into and out of the sieve-tube, however, is presumably confined to crystalloid N. On this view, nitrogen should move from bark to wood, and vice versa as crystalloid N; and since the inter-conversion of crystalloid and protein N in the wood is relatively slow, the response shown to ringing in the wood should be mainly crystalloid N. It will be noted that in the two experiments (2 and 3), in which the crystalloid N in the wood was determined, the response was, in fact, mainly crystalloid N.

As to the crystalloid N fractions which take part in the radial movement, into and out of the sieve-tube, residual N may be of some importance, since it does not appear to share in the rapid changes (of crystalloid N to protein N) which we have observed in the bark.

As already noted, radial spread from the sieve-tubes into the other tissues of the bark appears to be greater for nitrogen compounds than for sugars. This radial leakage must diminish the rate of spread of concentration changes along the sieve-tubes, and it is interesting to note (2) that nitrogen transport does appear much more subject to damping than the transport of carbohydrates.

SECTION 4. SUMMARY.

A. *The Crystalloid N-Protein N Relation in Bark, Wood and Leaf.*

1. Conversion of crystalloid N to protein N, and vice versa, in the bark may be rapid compared with the rate of longitudinal transport of nitrogen.

Without appreciable alteration in total N content, the proportion of crystalloid N may alter very considerably in a short time. This marked lability is not shown by the protein and crystalloid N of wood and leaf.

2. As to the factors affecting the ratio of crystalloid N to protein N in the bark:

(a) Decrease in sugar concentration causes a conversion of protein to crystalloid N. There is a high negative correlation in the bark as a whole and in the inner and outer halves, separately, between sugar concentration and proportion of crystalloid N. (A similar association appears in the wood.)

(b) A decreased hydrogen ion concentration is associated with an increased proportion of crystalloid N, but the correlation is not statistically significant.

(c) Desiccation causes a conversion of crystalloid N to protein N, but the concentration of crystalloid N does not diminish. All the crystalloid N fractions seem to be involved in this conversion. (In the wood this effect of desiccation was not found.)

(d) A very rapid fall in the proportion of crystalloid N is generally found when the initial value is much above the average. This type of change, which may be characterized as an 'adjustment change', is independent of sugar concentration and of moisture content. Asparagine and amino-acids are the principal crystalloid N compounds involved in the conversion, residual N showing very little change. These rapid adjustment changes have not been observed in the wood or the leaf.

3. This lability of protein and crystalloid N is more marked in the inner and middle zones of the bark than in the outer zone, and is probably, therefore, of particular importance in the sieve-tubes.

4. These observations suggest that all the nitrogen fractions, including the labile protein, contribute to longitudinal movement within the sieve-tube, but that movement into and out of the sieve-tube may be restricted to some crystalloid N fraction (possibly residual N), which does not take in the rapid 'adjustment changes'. Otherwise these changes would produce violent disturbances in the rate of transport of nitrogen about the plant.

B. *Response to Ringing.*

1. Accumulation of nitrogen, not only in bark and wood, but also in the leaves above a ring is definitely established. At the same time further confirmation is obtained of the spread of diurnal changes in total N content from leaf to bark. These observations support the general conception of a gradient basis for nitrogen transport.

2. The response of protein and of crystalloid N in four ringing experiments may be summarized as follows:

(a) In two of the experiments there were rapid adjustment changes in

the proportion of crystalloid N. In one case the response was mainly crystalloid N, in the other mainly protein. The type of response observed (protein or crystalloid N) would appear to depend on the mean level maintained by the proportion of crystalloid N during the interval between the time of ringing and the time of observation.

(b) In the other two experiments rapid adjustment changes were absent. The response was mainly crystalloid N in the first case, when the observations were made shortly after ringing, and mainly protein in the second, when observations were begun only after an interval of a day. This may be due to the effect of increased sugar concentration on the protein-crystalloid N ratio.

3. The crystalloid N response is mainly a response of asparagine.

4. Radial spread from the sieve-tubes into the other tissues of the bark and into the wood appears to be relatively greater for nitrogen than for sugars.

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The Experimental Cultivation of the Gametophytes of *Hymenophyllum pulcherrimum*, Col. and of *Trichomanes reniforme*, Forst. f.

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With thirty-two Figures in the Text.

INTRODUCTORY.

IN the following paper an account is given of the experimental cultivation from the spore of the gametophytes of two species of filmy ferns, viz. *Hymenophyllum pulcherrimum* Col., and *Trichomanes reniforme* Forst. f., both of which are endemic to New Zealand. The sequence in development of the gametophyte of the former is described up to the stage at which the ribbons are from eight to ten millimeters in total length, and twenty cells in width at the forward end. They have taken about three years and a half to reach this stage, and although sex organs have not yet been formed, the gametophytes are large enough to be compared directly with mature ribbons of the same species, bearing sex organs and sporelings, which the writer has collected in the field. A complete account of the development of the gametophyte of this species can thus be given with respect to its general structure.

The *T. reniforme* culture is, at the time of writing, just two years old, and although the gametophytes are not far enough advanced to show what their mature form will be, it is evident that they present an interesting variation from the form hitherto considered as normal for this genus. A short account of the earlier stages of development in this species has therefore been included.

Sufficient is known of the gametophyte generation in filmy ferns to indicate that in the genus *Trichomanes* it takes the form of a richly branched filament with both erect and creeping branches, and in *Hymenophyllum* of an irregularly branching ribbon. The cordate habit of growth, such as is characteristic of so many other fern genera, has not been found

in any filmy fern. It must be said, however, that our information, with respect to the gametophyte in this fern family, is still very incomplete. It relates to only a few of the many species of these two widely distributed genera.

The chief published account of the experimental cultivation of the gametophytes of filmy ferns is that of Goebel (4), written as long ago as 1888. Spores of one or two species of both genera were sown by him, and after eight months the gametophytes were still very small, and had not formed sex organs. Germination was found by him to take place quite readily, but subsequent to the initial stages in the formation of the primary filament, development was very slow. This he ascribes to 'the very sparse development of rhizoids which here are evidently only attaching organs' (loc. cit., p. 93). In the case of the young *Hymenophyllum* gametophytes, he describes the early setting in of a cell-surface by the activity of a 'two sided' apical cell.

In the same paper, Goebel summarizes the results obtained by Mettenius in 1864 from the sowing of spores of *H. tunbridgense* Smith, and of certain other species. The present writer has not had access to Mettenius' original paper. Goebel states that these cultures 'seem to have yielded only slight results: one and a half year old prothalli of the named *Hymenophyllum* species had attained a leaf-like spread half a line in length, and one-twentieth of a line in breadth. Recognizable sex organs were not formed' (loc. cit., p. 90).

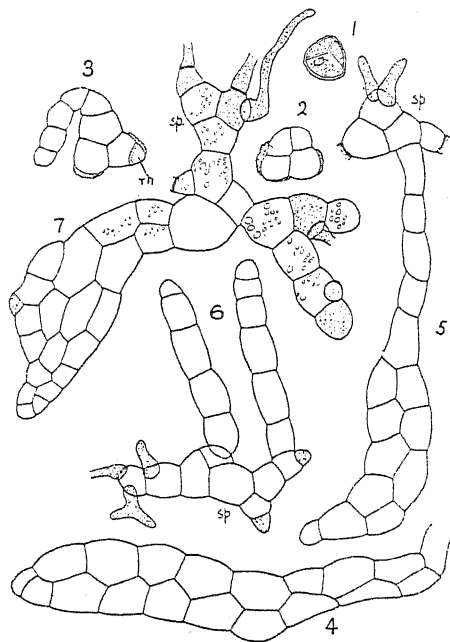
Bower (1) observed germination of the spores of *T. pyxidiferum* Linn. in the sorus, in the case of plants of this species kept in the filmy fern house at Kew Gardens, and found that the details of the early development corresponded closely with what Goebel had described for other species of *Trichomanes*. Others also, for example, Campbell (3, p. 373) and Sadebeck (7, p. 93) have published figures of the earliest stages of spore germination in species of both genera. So far as the present writer can ascertain, however, the development of the gametophyte has not been followed in experimental cultures in any filmy fern beyond a comparatively early stage.

CULTURE METHODS EMPLOYED.

Well-grown clumps of the tufted *H. pulcherrimum* were suspended in a Wardian case in baskets of fine mesh wire netting, the clumps of humus being packed with well-boiled sphagnum. The spores were thus shed naturally upon plates and pots placed beneath. These plants were originally set up in position just over five years ago, and have produced a succession of healthy new fronds.

At first white porous plates, standing in shallow dishes in which a little water was constantly kept, were used on to which the spores could fall. The spores germinated abundantly on these plates, and the very

young gametophytes could clearly be distinguished upon the white background. Further growth, however, was exceedingly slow, the gametophytes after three years having advanced no further than the stages shown in



FIGS. 1-7. *Hymenophyllum pulcherrimum*, Col. Culture gametophytes. 1. Ungerminated spore. $\times 200$. 2. Unusual segmentation of spore. $\times 200$. 3. Usual type of germination of spore. $\times 200$. 4 and 5. Passage of primary filament into a flattened cell-surface, owing to activity of a 'two-sided' apical cell. $\times 200$. 6 and 7. Early initiation of lateral branching. Browned cells and copious oil droplets in basal part of Fig. 7. $\times 200$.

Figs. 4-7. This was no doubt largely due to the fact that the flat surface of the plates was continuously wet with a film of stagnant water. Moreover, colonies of unicellular algae began to spread over the plates. For the greater part of these three years, the young gametophytes kept healthily green, but showed no tendency to grow erect except when they happened to spring from a particle of humus or from an old sporangium. Finally, they began to show obvious signs of browning and decay, and growth had clearly ceased.

Fifteen months after the culture was first set up, an inverted unglazed garden pot, tightly filled with sphagnum, both pot and moss having been well sterilized by boiling, was placed in the case, standing in a dish of water. The spores have germinated freely on the upper surfaces and on the sides of the pot, and the gametophytes have grown faster and more healthily here than on the plates. At the time of writing, three years and nine months after the pot was first placed in position, there is a strong, healthy,

turf-like growth of gametophytes, standing up in hundreds more or less erect from the surface. The largest of these are up to ten millimetres in length, the ribbons being commonly from 15–20 cells broad behind the growing ends. That shown in Fig. 12 is $6\frac{1}{3}$ rd millimetres long. With respect to the age of these gametophytes, it can be assumed that spores were shed upon the pot within a short time of its being placed in the case, so that the largest will probably be a full three and a half years old.

Spores of *T. reniforme* were also allowed to fall naturally upon porous plates from living plants in a Wardian case, and, as in the case of *H. pulcherrimum*, they germinated freely and have in two years developed to the stage shown in Figs. 30 and 32.

It was necessary, in order that the spores should be freely shed, that the fronds should be kept fairly dry, so that with both species the plants were only taken out of the cases for a hosing whenever the fronds began to show obvious wilting. The cases have been kept in a glasshouse whose sides were painted green, and have been taken each year into a cooler room during the height of the summer.

The possibility that abnormally moist conditions in Wardian cases may induce a filamentous habit of growth in the ribbon gametophytes of *Hymenophyllum*, is suggested by the fact that large numbers of rapidly growing gametophytes of a markedly filamentous form were, on one occasion, observed in and around some of the sori on the fronds of *H. pulcherrimum*. One of these is shown in Fig. 8. The gametophytes rising up from the sphagnum in the drainage hole in the bottom of the inverted pot were also of this form during their earlier stages of growth.

THE DEVELOPMENT OF THE GAMETOPHYTE OF *H. PULCHERRIMUM* COL.

The primary filament. Spores of this species of *T. reniforme*, dissected out from sporangia on the frond, were commonly found, as is well-known to be the case in filmy ferns generally, to have already germinated up to the three-celled stage by the formation of three radiating walls.

The formation of the primary filament in *Hymenophyllum* has been described by Goebel (4), so that it is unnecessary to go into this in detail here. This filament is usually up to five or six cells long (Figs. 5, 17), but may be, under exceptional conditions, considerably longer (Fig. 8).

While it is often the case in this species that further development proceeds as a direct continuation from the primary filament (Figs. 5, 8, 17), the main axis of growth not infrequently arises on the latter as a lateral branch (Figs. 6, 9). It is evident that there is here no constant sequence in development, such as is found in fern gametophytes of the cordate type, and this is further shown in the proneness of the *Hymenophyllum* gametophyte to

adventitious branchings from the main axes which is to be seen throughout its life.

The apical meristem. Sooner or later, by the formation of oblique intersecting walls in the terminal cell of the filament, an apical cell with two cutting faces is set apart from which further segmentation proceeds (Fig. 4). Commonly the gametophyte maintains the forms in which it is two cells wide for a considerable length (Figs. 4, 9, 17). That there is considerable variation, however, at this stage of the development is evident from Figs. 5, 7, and 8.

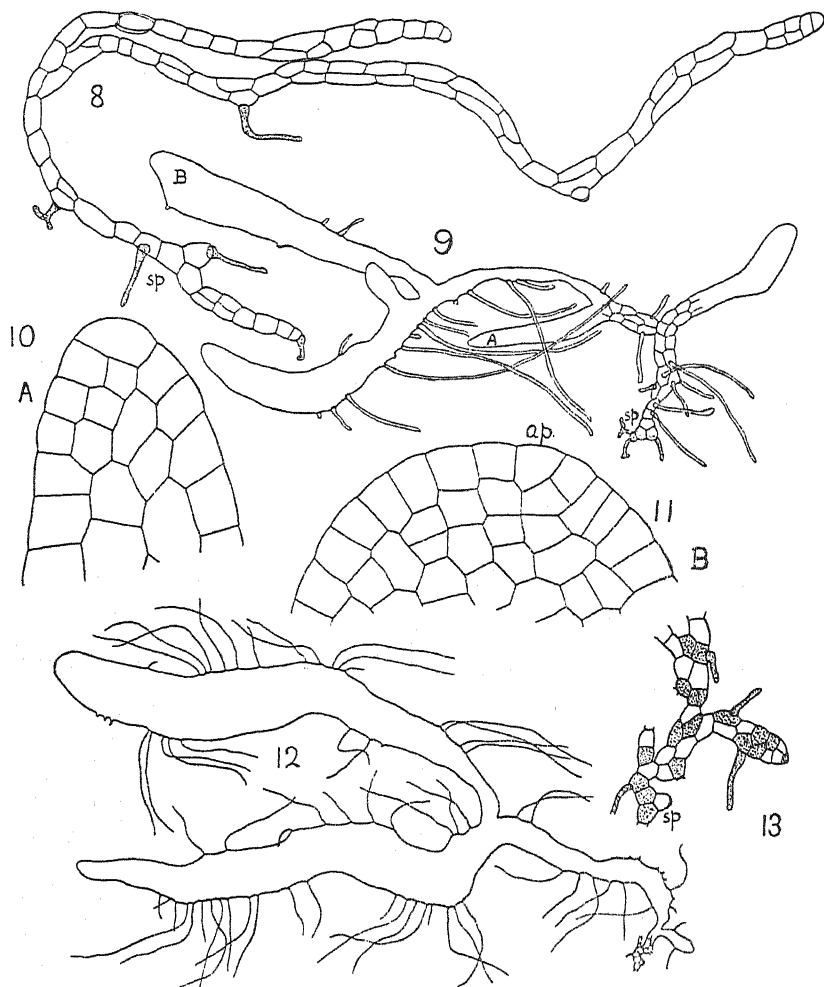
The main axis progressively broadens owing to the subdivision of the segments at or close behind the apex (Figs. 9-12). The single apical cell condition is maintained for a considerable time in the further development. It is also found in the narrower adventitious branches of mature gametophytes of this species collected in the field, as is known to be the case also in such branches in other species of *Hymenophyllum*. There is no indication in *H. pulcherrimum*, nor in any of the other species of *Hymenophyllum* whose adult gametophytes the writer has examined, of the more rapid forward growth of the segments beyond the actual apex, such as leads to the familiar cordate form in so many other fern genera. The apex remains rounded (Fig. 11) up to the latest stages in the development leading to the ribbon form of gametophyte so characteristic in *Hymenophyllum*.

In the broadest branches of the oldest culture gametophytes (Fig. 12), the single apical-cell has been superseded by a marginal meristem of equivalent cells, and from such a meristem all further extension in length in these broad ribbons takes place. The gametophyte as a whole takes the form of a gradually broadening and branching ribbon which remains but one cell-layer thick.

Branching of the ribbon. The gametophyte is prone throughout its life to the formation of adventitious lateral branches (Figs. 6, 9, 16). Such a lateral branch has usually a very localized origin, arising sometimes from a marginal cell of the parent axis (Figs. 9, 16), thenceforward broadening more or less rapidly owing to the early formation of its own apical-cell. Even in mature gametophytes lateral branches may sometimes be seen to show an extremely narrow base (Fig. 21). Occasionally it was observed in the culture gametophytes that, owing to the formation of a strong lateral branch close behind the apex of the main axis, further growth of the latter had ceased, its meristem having passed over into permanent tissues bearing rhizoids (Fig. 16). That this does not always happen, however, is clear from Figs. 9 and 12.

Not only may lateral adventitious branches be formed, but the apex of a main axis may fork into two equally strongly growing apices (Fig. 17). In Figs. 19 and 20 is shown a mature gametophyte collected in the field in which the manner of forking can be seen. Here the central marginal

region of a strong branch, marked *br.* in Fig. 19, has passed over into permanent tissue, while the tissue to right and left of it, has remained meristematic, with the result that two equivalent growing apices have begun to

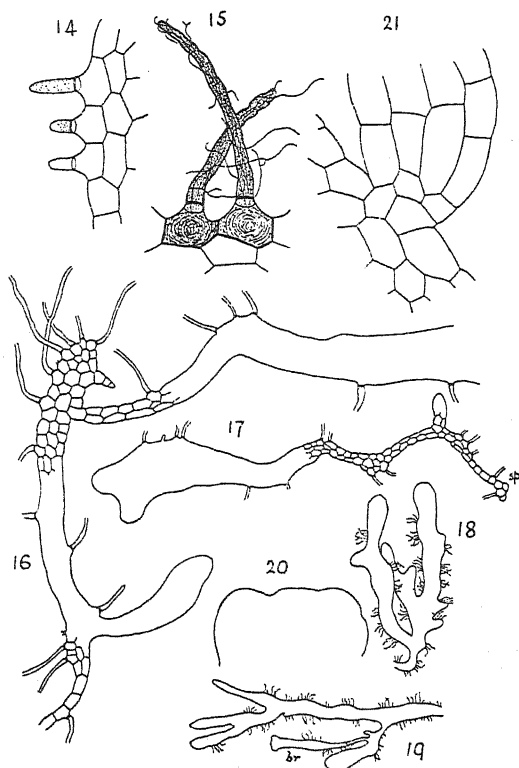


FIGS. 8-13. *Hymenophyllum pulcherrimum*, Col. Culture gametophytes. 8. Abnormal extension of filamentous stage; the two long terminal branches were erect in habit. $\times 70$. 9 and 12. Typical complete examples of older gametophytes, showing original spore end, lateral branching marginal position of rhizoids, and broadening ribbons. Fig. 9 $\times 22$; Fig. 12 $\times 14$. 10 and 11. Apex of two of the branches (marked A and B) of gametophyte shown in Fig. 9. $\times 275$. 13. Details of spore end of gametophyte shown in Fig. 12. $\times 42$.

extend forward beyond the original apex which now lies in a hollow between them. Whether or not the apical forking of the *Hymenophyllum* ribbon is always initiated in this way cannot here be stated.

As a further illustration of the adventitious branching to be met with in the *Hymenophyllum* gametophyte, it may be mentioned that the writer

has observed that old, brown, decaying ribbons of *H. villosum* Col., collected in the field, not infrequently may be seen to be giving rise to actively growing, healthy lateral branches. Such a branch originates from a small



FIGS. 14-21. *Hymenophyllum pulcherrimum*, Col. 14-17. Culture gametophytes. 18-21. Mature gametophytes collected in the field. 14. Origin of rhizoids close behind growing apex of ribbon, showing early browning, and thickening of basal wall before infection by fungus. $\times 140$. 15. Older rhizoids showing fungal infection and fungal coil in the rhizoid-bearing-cells. $\times 210$. 16. Showing cessation of growth of main primary axis of gametophyte owing to lateral origin of large secondary axis from a single marginal cell. $\times 57$. 17. Branching of main axis of gametophyte by the apparent forking of the apex. $\times 35$. 18. Portion of typical mature gametophyte which bore sex organs, collected in the field. Basal region decayed. $\times 4$. 19. Another mature field gametophyte. $\times 4$. 20. Apex of one of the branches (br.) of gametophyte shown in Fig. 19, showing initiation of two growing points at the apex. $\times 35$. 21. Origin from two marginal cells of the same lateral branch (br.) of the gametophyte shown in Fig. 19. $\times 210$.

group of a few cells which have remained healthily green in striking contrast to the rest of the ribbon which is brown and apparently dead. The branch begins as a small bud, or even as a short linear filament, and rapidly widens *in situ* into the usual ribbon form.

The rhizoids and fungal infection. On the primary filament the rhizoids have a very stunted form, and early turn brown. It seems clear that they do not retain the absorbing function for long. Subsequently formed rhizoids arise on progressively older parts of the gametophyte,

usually in groups, along the margins of the ribbons. These show fungal infection, one or more hyphae penetrating the wall of the rhizoid either before or after browning begins (Figs. 14, 15). According to Goebel (4, p. 93) the rhizoids of the *Hymenophyllum* gametophyte are to be regarded chiefly as attaching organs. The writer has observed with respect to the mature gametophytes of *H. pulcherrimum* and of various other species collected in the field, that the ribbons usually either stand away from the substratum or overlies one another in large numbers owing to the copious development of lateral branches, so that it is only on the oldest basal portions of the ribbons that the rhizoids are actually in contact with the soil. On the more or less erect growing culture of gametophytes of *H. pulcherrimum*, groups of rhizoids continue to arise on the forward part of the ribbons, but they are far too short to reach the substratum. In the case of the adult filamentous gametophyte of *S. strictum* Menz, collected in the field, it was found that rhizoids of quite a functionless character are present. They are peg-like and of the usual brown colour, and are borne both on the creeping branches and also on those which are erect and green.

Where the gametophytes of *H. pulcherrimum* are thickly clustered on the culture pot, the fungal hyphae spread over them like a diffuse delicate cobweb. In the older basal parts of the ribbons, the rhizoid-bearing cells are usually infected as well as the rhizoids themselves, and contain a dense hyphal coil in the cell interior (Fig. 15). No accumulation of food reserves could be detected in these nor in the cells in their immediate neighbourhood. In no case was the fungus seen to extend outward from them into the surrounding cells of the gametophyte.

It would seem that the fungal infection is of only minor importance in these gametophytes. It is not the cause of the loss of the absorbing function of the rhizoids, nor does it seem to affect the vitality of the gametophyte either beneficially or otherwise.

THE MATURE GAMETOPHYTE OF *H. PULCHERRIMUM*.

The writer has found these in large numbers in one locality associated with mature sporophytes on a clump of humus on a forest tree. Many young sporelings in practically all stages of development were present in the same clump, the youngest of these being still attached to their gametophytes. The continuous sequence in frond development which could be traced made it certain that these gametophytes belonged to *H. pulcherrimum*. Moreover, no other species of filmy fern was present in the vicinity of this clump. An account is here given of these field gametophytes since by this means the sequence in development in size and form of the gametophytes of this species, described above from culture material, can be completed.

None of these mature gametophytes possess the basal region of the original axis of growth intact, the oldest parts being always much browned and in a decaying condition. This is the usual state in which *Hymenophyllum* gametophytes are found in the field. The progressive decay of the oldest regions, as the forward parts of the ribbons grow on, was observed also to have begun in some of the largest of the culture gametophytes described above. Filmy fern gametophytes, so far at least as some New Zealand epiphytic species are concerned, have been observed by the writer to possess remarkable powers of withstanding drying. This is referred to again below. It is quite possible, therefore, that their natural development in the field may take quite as long as it does in the culture experiments, and may be still further prolonged.

Many of the mature gametophytes are much branched, leading to an overlapping rosette habit of growth. Undoubtedly, a good many of those found had originated as branches which had become detached from older axes. Some of them are up to seventeen millimetres in length (Figs. 18 and 19), the main branches being from twenty-five to thirty-five cells broad. They can thus be compared very closely with the oldest of the culture gametophytes already described, whose ribbons are almost as broad although not as long.

In most of them, antheridia were present around the margins, in some cases in great abundance. These sometimes occur spaced out on marginal permanent tissue, but not infrequently they are in distinct groups on meristematic marginal tissue which is more than the usual one cell-layer in thickness. Groups of old archegonia were also observed in a number of the gametophytes seated on such marginal meristematic cushions, antheridia being intermingled with the archegonia.

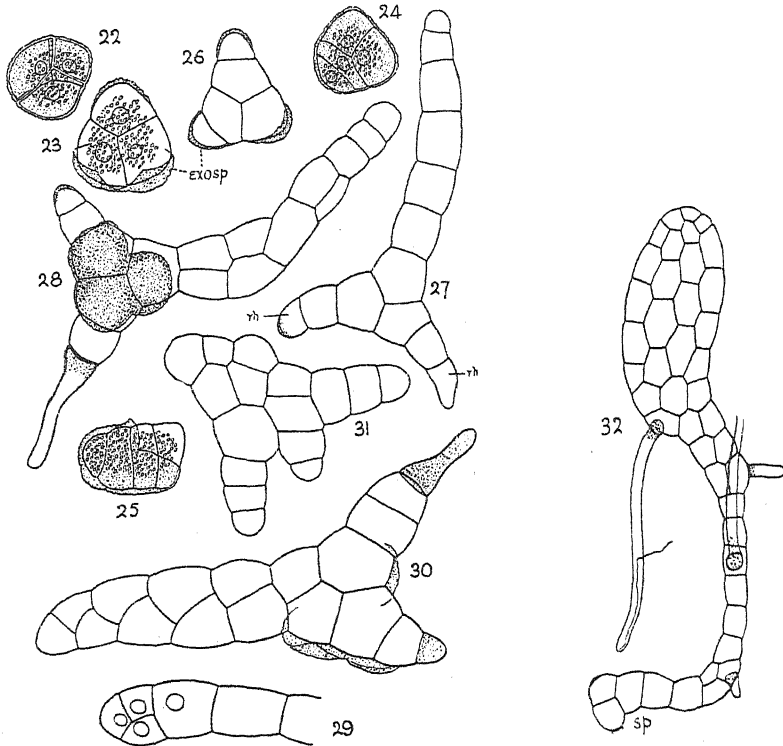
Branching in these mature gametophytes takes place as described above, viz., either by the forking of the apex of a ribbon or by the development of adventitious branches, the latter arising commonly from a very restricted portion of the margin of the parent ribbon (Figs. 19 and 21). It is such branches as these which can readily become detached and so constitute independent gametophytes.

Gemmae were present in abundance around the margins of the growing apices of a large proportion of these gametophytes in much the same way as has been described by other writers in other species of *Hymenophyllum*. Large numbers of detached gemmae were also found in the humus in which the gametophytes were growing.

Probably the culture gametophytes (described above) are still too small to bear sex organs or gemmae, but it is quite possible that the artificial conditions of growth are responsible for the absence at least of antheridia. It is hoped that by the extension of this culture experiment the sequence in development of the sex organs and of the embryo will be traced.

EARLY STAGES IN THE DEVELOPMENT OF CULTURE GAMETOPHYTES
OF *TRICHOMANES RENIFORME* FORST. f.

According to Sadebeck (7, p. 93) and Mettenius (quoted by Goebel, 4, p. 92), the species of *Trichomanes* differ from those of *Hymenophyllum* in



FIGS. 22-32. *Trichomanes reniforme*, Forst., f. Culture gametophytes. 22-24. Germinating spores taken from sporangium, showing chloroplasts in the cells. $\times 315$. 25. Abnormal segmentation of spore. $\times 315$. 26-28. Further stages in germination of spore after shedding. $\times 315$. 29-30. Development of a cell surface owing to activity of a 'two-sided' apical cell. $\times 315$. 31. Irregular early development. No rhizoids formed. $\times 315$. 32. The oldest stage in development found in the culture. $\times 160$.

the fact that the spores of the former on germination divide in such a way as to cut off three papilla-like corners from a centrally placed cell. Goebel (loc. cit.) describes this as taking place in *T. diffusum* Bl., but states explicitly that such is not the case in certain other species of *Trichomanes*. It is evident, therefore, that in some of the species of this genus the initial segmentation of the spore does not differ from what takes place in *Hymenophyllum*, and it can be stated here that this is the case in *T. reniforme* also.

The primary filament formed from the spore is more or less similar to that in *H. pulcherrimum*, except that in *T. reniforme* a large proportion of

the young gametophytes examined showed at first a decided tendency to the filamentous growth of all three primary cells (Figs. 26 and 27). In some cases two of these primary arms continue to develop for a time equally strongly, but sooner or later growth becomes restricted to one arm only. This main filament becomes usually no more than five or six cells long (Fig. 27), although occasionally such young gametophytes up to ten or twelve cells in length were met with (Fig. 32). A few instances of early abnormal development were also observed (Fig. 31).

In *T. diffusum*, according to Goebel (4), the young gametophyte is completely filamentous, and increasing complexity is attained only by copious branching. This seems to be the case in species of *Trichomanes* generally, the mature gametophyte having been described by various observers as a much branched filament without cellular expansions. The present writer has found that this holds also for the three New Zealand species, *T. Colensoi* Hook. f., *T. strictum* Menz., and *T. elongatum* A. Cunn., whose mature gametophytes he has collected in the field.

In *T. reniforme*, however, the primary filament early passes over into a ribbon by the formation of a 'two-sided' apical-cell, just as takes place in the developing gametophyte of *H. pulcherrimum* and, presumably, in species of *Hymenophyllum* generally (Figs. 29 and 30). Further development is exceedingly slow, but it is clear that a typical slowly broadening ribbon is formed. After eighteen months, a considerable number of the culture gametophytes were found to have reached the stage shown in Fig. 32.

The writer has not succeeded in identifying the mature gametophytes of *T. reniforme* in the field.

DISCUSSION.

From the comparative study of existing and of fossil ferns, it appears that certain characters in the Hymenophyllaceae may be regarded as relatively primitive, and others again as due to specialization to a more or less hygrophilous mode of life (2, vol. ii, ch. 27). The following remarks relate to the status of the two types of gametophyte characteristic respectively of *Hymenophyllum* and of *Trichomanes*.

Professor Bower is of the opinion (2, vol. i, p. 281) that the strap-shaped gametophytes of *Hymenophyllum* 'may well be held to be flattened derivatives of a filamentous type'. He has shown (1) that on aposporous gametophytic filaments which arise from the fronds of *T. alatum*, flat cell surfaces are sometimes formed by repeated longitudinal cell division in some of its branches. Goebel refers to the fact that Mettenius found the same kind of cell-surface formation on the filamentous gametophytes of *T. incisum* and *T. sinuosum* (4, p. 109). Bower regards this as evidence of the evolution of the ribbon type of gametophyte from the filamentous, and Goebel also at

one time held the same opinion (loc. cit.). Sadebeck (7), in his short account of the gametophyte of the Hymenophyllaceae, is evidently referring to these cases when he states that in *Trichomanes* the filament may take a 'higher form' by passing over into a ribbon-like surface by the longitudinal division of its cells. It seems to the present writer that this phenomenon belongs to a different kind of cell-surface formation from that which takes place from a 'two-sided' apical-cell in the developing gametophytes of *Hymenophyllum* and of *T. reniforme*, and that it is the one which cannot properly be considered as pointing to the phylogenetic origin in this family of the cell-surface from the filament.

Goebel later (5, pp. 963 and 1191) contemplated both these types as merely variants of a growth-form which is common to all homosporous leptosporangiate ferns, and one which in its most complete form attains the well-known heart shape after passing through filamentous and strap-shaped stages. Thus, according to his view, the *Hymenophyllum* and *Trichomanes* types are to be regarded not as having actually been derived the one from the other, but as representing 'checked' types, which remain in a youthful stage inasmuch as neither attains the complete form possible.

Reference must be made to the fact that filamentous alga-like gametophytes are found also in at least three species of *Schizaeae*. Here, however, the filaments show clear specialization to mycorrhizic nutrition, and Bower, in his latest statement (2, vol. ii, p. 246), mentions as a possibility that the fungal infection may be the direct cause of the filamentous habit. This could not be said of *Trichomanes*, where the infection is of a very slight nature.

Campbell (3, p. 372) has expressed the view that the peculiar characters of the gametophytes in Hymenophyllaceae are adaptive rather than primitive. The facts brought forward in the present paper seem to accord well with this view. The developing gametophyte of *H. pulcherrimum* is clearly a very plastic structure, as is shown in its lack of any regular sequence of growth, and it may be assumed that this is the case in other species of *Hymenophyllum* also. The fact that the young gametophytes of this species were found to develop a markedly filamentous habit of growth under specially moist conditions makes it quite conceivable that the *Trichomanes* filament has actually arisen from an ancestral ribbon as an advanced feature of that specialization which the family as a whole undoubtedly shows to an hygrophilous mode of life. Again, throughout the family, the rhizoids formed on the gametophyte are to a greater or less extent non-absorbing organs. This is most marked in *Trichomanes*, where they are either wholly absent or are represented (as in *T. strictum*, quoted previously) by mere rudimentary pegs. In the case of *Hymenophyllum*, water absorption apparently takes place over the entire surface of the ribbon, and in *Trichomanes*

by means of the creeping branches of the filament. This is probably best interpreted as being an adaptation to an especially moist environment, and is in accordance with the view that the *Trichomanes* gametophyte has become thus modified to a greater extent than has that of *Hymenophyllum*.

These facts can, of course, also be interpreted in the way that Goebel has done, without bringing in the question of an actual phylogenetic derivation of the one type of gametophyte from the other. A further fact, however, which seems to provide evidence that the ribbon type is the ancestral one for the Hymenophyllaceae as a whole, is that in *T. reniforme* the gametophyte is not the usual *Trichomanes* branching filament but is ribbon shaped, this form being attained in development from the spore in the same way as in the genus *Hymenophyllum*. That this possesses phylogenetic significance seems to follow from the fact that *T. reniforme* has been considered on the basis of its large spore-output per sporangium, its stelar character, and its several-layered lamina, to occupy 'together with some large species of *Hymenophyllum*, a central position in the family, from which divergent lines of specialization may have radiated' (2, vol. ii, p. 248). This at once suggests that in the ribbon form of its gametophyte, *T. reniforme* is more primitive than are the other species of *Trichomanes*, the modification of the older ribbon into the much branched filament of the latter species representing one of those lines of specialization which have proceeded from the central stock of the family. In this connexion, it should be noted that *T. reniforme* is by no means specially hygrophilous, since it occurs usually in a mid-epiphytic station in the forest, and is the most widely ranging of all the New Zealand species of *Trichomanes*.

It may be useful, finally, to place the facts concerning the gametophyte generation of the Hymenophyllaceae over against those relating to the habit of life of the family as a whole. A point of especial significance is that the frond lamina, practically throughout the family, is only one cell-layer in thickness, the most reasonable explanation of this being that the family, at an early stage in its evolution, became specialized to an hygrophilous mode of life. Absorption of water can take place over the whole surface of the frond lamina and of the gametophyte, and, probably as a result of this, there has been a tendency towards the reduction of root absorption and of stem vascular tissues in the sporophyte, and, in the gametophyte, of absorbing rhizoids.

A second outstanding feature in the biology of the family is the marked ability shown by the more typically mid- and high-epiphytic species to withstand excessive drying, and this in spite of the 'filmy' nature of their frond lamina. This has been previously dealt with by the writer (6) in a study of the twenty-six New Zealand species. As regards the sporophyte generation this is seen in the well-known ability of the fronds of many

species to recover after extreme wilting as soon as they are wetted : in the frond stunting with overlapping of the pinnae, and in close mat formation, in the high epiphytes : in the development, in a number of species of a copious frond tomentum ; and in such species as *H. malingii* Metten., in a most remarkable modification of the frond lamina for the absorption and holding of water. The gametophyte, also, of some of these epiphytes has been observed by the writer to possess the same power of withstanding the effects of drying. For example, *H. rarum* R. Br. is one of the last of the species to show wilting of the fronds, and during exceptional dry periods its gametophytes also have been observed to be quite unaffected when the short moss in which they were growing was dry enough to be powdered in the hands. *H. villosum* Col. is an abundant epiphyte on the mountain *Nothofagus* in the South Island of New Zealand in situations such as Arthur's Pass where dry periods are not uncommon, but its gametophytes can be found in the short moss on the bark of almost every tree in such places. Seeing that these species possess the usual one-layered frond lamina, it would appear that they have become specialized to more or less exposed conditions as a more recent modification of an older hygrophilous habit.

A third general feature in the family to be noted is that the species of *Trichomanes* are hygrophilous to a much greater extent than are those of *Hymenophyllum*. The former in New Zealand are more restricted to wet forests than are the latter, and, except in the case of *T. reniforme*, they occupy always a terrestrial or lower epiphytic station. Whether or not this holds for species of *Trichomanes* elsewhere, cannot be definitely stated, but that this is the case seems to be indicated in the literature. The writer has observed in the case of three New Zealand species, *T. Colensoi* Col., *T. strictum* Menz., and *T. elongatum* A. Conn., that the filamentous gametophytes, which are to be found only in specially sheltered places on the forest floor, are quite incapable of recovery after even a moderate degree of drying, and, so far as can be judged from field observation, seem to have a very much shorter life than do those of *Hymenophyllum*.

On various grounds, *T. reniforme*, *H. dilatatum* Swartz, an *H. scabrum*, A. Rich. have been regarded (2, vol. ii, p. 248) as probably representing best the ancestral stock of the family. When compared with the other New Zealand species as regards ecological behaviour, they must certainly be regarded as representing the least specialized section of the family. The peculiar-shaped form of the adult frond of *T. reniforme* must be conceded as a modification of the more normal frond type, a modification indeed which, by allowing of inrolling, enables this species to range somewhat more widely than the other two mentioned, with which it usually associates, but that this is probably quite a recent feature in this particular species, and hence one which does not affect the present argument is indicated by the fact that the first two or three fronds in the sporcling are pinnatifid, and, indeed

very little different in form from those of such species as *H. dilatatum*. Again, the fact that these three species possess a frond lamina several cell-layers in thickness can scarcely be interpreted otherwise than as the retention of an ancestral character. They are less capable of ranging from a low or mid-epiphytic station in the forest than are a number of the other New Zealand species which do not possess that type of lamina, so that the several-layered lamina could hardly be regarded as an adaptation to a more exposed habit of life recently developed in them.

On an ecological basis, therefore, the New Zealand species seem to fall into three main groups. The central and least specialized of these groups comprises the three species just discussed, *T. reniforme* possessing, as indicated by the culture data described in this paper, along with the two species of *Hymenophyllum*, the ribbon type of gametophyte. Of the two other groups, one comprises species which are restricted to wet forests, and there to a terrestrial or at the most to a low epiphytic station.

Of the members of this group the species of *Trichomanes* are undoubtedly, as a whole, more hygrophilous than those of *Hymenophyllum*, a fact which accords well with the suggestion that the filamentous *Trichomanes* gametophyte has been evolved as an extreme adaptation to this habit. The third group comprises the mid- and high-epiphytes, and these belong only to the genus *Hymenophyllum*. If the gametophyte of these latter species has undergone any specialization in accordance with the more exposed habit of life, it has not been in any modification of the typical ribbon form, but rather in the development of special cytological characters which enable it to withstand a considerable degree of drying.

SUMMARY.

1. The development of the ribbon-like gametophyte of *H. pulcherrimum* Col. is described in detail from culture material, up to a fairly advanced stage attained after about three and a half years' growth. Although sex organs have not yet been formed on them, the largest of these gametophytes are shown to be comparable with mature gametophytes collected in the field, which are also described.

2. A description is given, from culture material, of the earlier stages in the development of the gametophyte of *T. reniforme* Forst. f., from which it is clear that this particular species of *Trichomanes* is peculiar in that the gametophyte is not filamentous but passes over at a very early stage in its development into the form of a flattened cell-surface, in the same way as takes place in the gametophyte of species of *Hymenophyllum*.

3. The main facts relative to the filamentous and ribbon types of gametophyte, which have been brought forward both by previous writers and in the present paper, are discussed with especial reference to the well-

known suggestion, previously made on evidence derived from the sporophyte generation, that *T. reniforme* belongs to a group of existing species which best represents the ancestral stock of the family. From these facts it is concluded that the filamentous type of gametophyte has arisen in the *Trichomanes* section of the family as a modification of the ribbon type in accordance with the adoption of a more hygrophilous mode of life by the species of this section.

4. An attempt is made to show that this conclusion accords well with the facts relating to the general ecology of the family as represented by the twenty-six New Zealand species.

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Observations on some Wound Reactions in the Aerial Stem of *Psilotum triquetrum*.

BY

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With twelve Figures in the Text.

THE material upon which the present observations are based was mostly derived from supplies for class use in the Nottingham collection. In addition, however, I received further stems, some of which showed wounds, from Mr. E. M. Cutting, M.A., and some living wounded material from the Director of the Royal Gardens, Kew, to both of whom I am much indebted. In dealing with work of this kind upon tropical plants the difficulty of obtaining experimentally wounded material is a distinct drawback, but I was able, in the present instance, owing to the good offices of Professor A. C. Seward, F.R.S., to have some experiments performed at the Cambridge Botanic Garden with a view to checking the observations made upon the remaining specimens.

The morphology of the aerial stem of *Psilotum triquetrum* is now so well known that a brief description of its salient features will suffice. It consists of a ridged, repeatedly dichotomizing axis bearing small scale leaves along its whole length. The epidermis is strongly cuticularized and is provided with stomata in considerable numbers. The cortex is divisible into three zones the outermost one, consisting of assimilatory parenchyma, being from three to six cells in width, the middle one of thick-walled cells, somewhat wider, and showing a varying degree of lignification, and a wide inner one of parenchyma limited by an endodermis of the primary type with a relatively broad Casparian strip. This endodermis is differentiated within 2 mm. of the tip of the shoot. The stele contains a core of sclerenchyma, surrounded by the xylem, which has a stellate outline with the exarch protoxylem occupying the points of the star. The xylem is, in turn, surrounded by parenchymatous tissue usually interpreted as phloem.

In the small ultimate branchlets both the cortical and intrastelar sclerenchyma are absent, the xylem forming a small diarch or triarch plate. [1, 3].

For purposes of description the wounds may be divided into two categories, namely, those involving the destruction of the apex, and those in which lateral injury of superficial or more deep-seated character has occurred. In both types the sound tissues in the neighbourhood of the injury, and for a distance beyond it, constantly show a marked discoloration of the walls, this ranging from a bright golden yellow to a sherry brown. The colour is readily removed from sections by warming in Eau de Javelle, and gives a greenish-black reaction with ferric chloride solution. Apart from this feature the two types of wound show considerable differences in their method of reaction, and will be described separately.

A. *Branches showing apical injury.*

The tip of a branch showing apical injury is generally dark brown in colour, and consists of dead and collapsed tissue, this forming a protective scab. As a rule, the cortical cells survive at higher levels than those of the stele, and form a hollow sheath, which, apart from radiating gum-filled cracks, consists of apparently healthy tissue, surrounding a brown disorganized plug of stelar elements. It is not uncommon to find isolated cells in this part of the cortex which have undergone one or two divisions suggesting an incipient local meristem. The radiating cracks in the cortex frequently extend for several millimetres downwards from the apex and, in the lower portions, the enclosed plates of parenchyma may show considerable evidence of meristematic activity, this ranging from cell-elongation to a whole series of transverse divisions. A diagram illustrating the disposition of a series of four such cracks in a stem is shown in Fig. 1 *a*, whilst a small portion of one of these is illustrated in Fig. 1 *b*. From this it will be seen that the main crack is frequently extended by subsidiary oblique and tangential ones, these enclosing little pockets of irregular meristem. In the case figured the endodermis has been penetrated, and the parenchymatous cells internal to it have undergone either elongation or elongation and division, whilst the endodermis itself cannot be identified in the region of secondary activity. The penetration of the endodermis is not essential, however, to the inducing of cell-division as in the majority of the specimens examined such penetration does not occur.

A further cortical modification in this type of wound, which, though not constant, is of frequent occurrence, consists of an alteration of the walls of the inner parenchyma. This alteration takes the form of a local thickening of the cells accompanied by the development of a dark brown colour, and may affect not only a large proportion of the cortical parenchyma, but also occasional cells in the thick-walled zone. Such cells are a normal feature of many stems, especially towards the base (1, 3, 9), where they form a narrow band one to three elements in width immediately beyond the endodermis, but their general distribution through the cortex appears to be

quite definitely associated with traumatic stimulus. The brown colour is generally considered to be due to phlobaphenes. It is removed by heating with fresh Eau de Javelle the bleached walls then giving a cellulose reaction with chlor-zinc-iodine (Artschwager's formula).

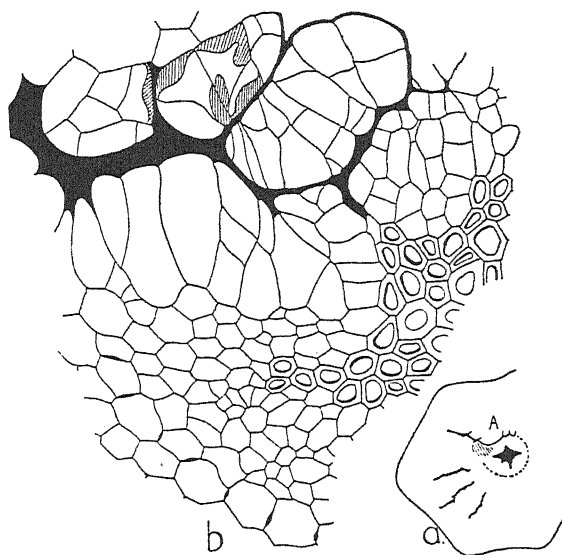


FIG. 1. (a) Diagram of part of a transverse section of the aerial stem of *Psilotum triquetrum* showing the positions of four cortical fissures ($\times 5$); (b) detailed drawing of a small portion of this stem taken from the region marked A in Fig. 1a. Note the elongation and division of both intrastelar and extrastelar parenchyma and the local loss of identity of the endodermis. $\times 250$.

The modifications in the stelar tissues, apart from collapse and death immediately below the seat of injury, offer some features of interest. The parenchymatous cells, as one would expect, occasionally show some signs of radial elongation and division. The tracheids which constitute the xylem are usually completely occluded by gummy matter, and, in addition, show a curiously irregular distribution quite unlike that of the uninjured stem (Fig. 2). The central core of sclerenchyma is often imperfectly developed, and is largely replaced by parenchymatous cells. As in the cortex irregular cracks, filled with gummy matter, are frequent, and cells showing the curious dark brown thickening of the walls, to which reference has been made above, occur scattered irregularly through the stele (Fig. 2). Their occurrence in the stele is quite unknown in the uninjured stem, and must be regarded as a wound reaction.

Among the class material at Nottingham is some purchased from Ceylon, and this shows a feature which is not exhibited by any of the remainder. This consists of a widespread deposit of gum which fills the whole of the cortical cells just below the injured apex, and affects large

patches of both cortical and intrastelar parenchyma for some distance downwards. This gum, which has a bright yellow colour, becomes dark brown on treatment with concentrated sulphuric acid, and stains a brownish

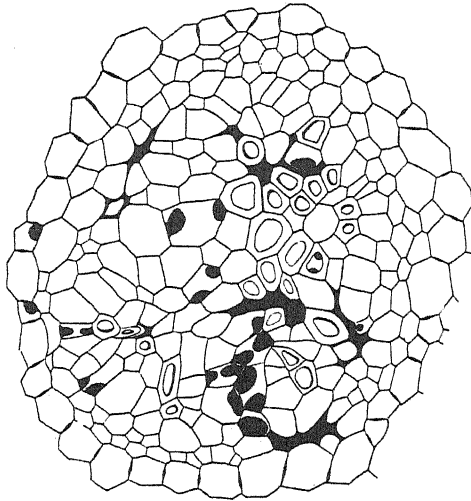


FIG. 2. Transverse section of the stele of an apically injured stem. Note the irregular distribution of the xylem, the partial suppression of the intrastelar sclerenchyma, and the presence of gum-filled fissures. The cells in which solid black patches are shown have local swellings on the walls coloured brown by phlobaphenes. $\times 250$.

red, with phloroglucinol, but appears to be insoluble in either boiling water or 10 per cent. caustic potash. It is produced by the degeneration of the cell walls, and it is quite common to find that this degeneration has caused local dissolution of the walls between adjacent cells so that the gummy contents fuse. In such cases small persistent strips of cell wall occur, giving a very characteristic appearance (Fig. 3). Occasionally the process proceeds further, the whole of the cell walls undergoing gummosis, and forming a gum pocket, surrounded by cells, which are apparently unaffected. In its mode of origin this gum closely resembles that produced in *Prunus* and *Citrus*.

Since both these genera include plants of great economic importance, the factors inducing this particular type of tissue degeneration have received considerable attention. Butler (2), who has fully reviewed the literature, and has made a detailed study of gummosis in *Prunus* and *Citrus*, concludes that its development results from any type of traumatism, provided that active growth is taking place, and that 'a superabundance of water' is present in the soil.

The fact that its occurrence is confined to a single batch of material in *Psilotum* rather suggests that its development is due to some environmental factor not present in the remaining cases, and this may well have been a high water-content of the substratum.

B. *Lateral wounds.*

The lateral wounds examined range from a mere grazing of the surface to relatively deep scars, but the depth of penetration of the tissues does not affect the nature of the traumatic response.

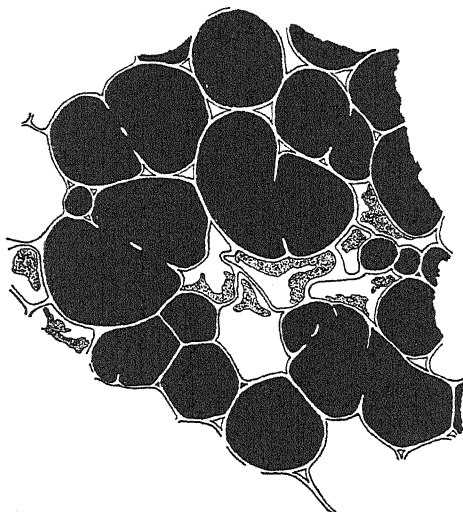


FIG. 3. Transverse section of a small group of cortical cells showing a fairly advanced stage of gummosis and consequent cell-fusion. $\times 333$.

As in the case of apical wounds, the material from Ceylon is characterized by local cortical gummosis, this taking the form of a superficial strip from two to six cells wide, consisting of gum-filled elements in various stages of breakdown (Figs. 4 and 8).

In the simplest cases the response consists of the death of the superficial cells and the formation immediately below these of a narrow pad of thickened cells, some three to five elements in depth. The thickening substance in the most superficial layer of modified cells consists almost entirely of cellulose, but this is replaced by lignin in the deeper layers. Where the response is more vigorous the zone of thickened cells may extend inwards so as to involve a much greater depth of tissue, and in such cases there is generally a superficial zone from one to three cells in depth, in which the thickening is wholly cellulosic, and in which the amount of thickening is greatest, this being succeeded by a deeper-seated zone in which lignification has occurred, the lignification becoming progressively more marked with greater depth.

An extreme case of this kind taken from a slender upper branch is shown in Fig. 4. Here it will be seen that the traumatic response has involved not only the cortical tissues, including the endodermis, but also the intrastelar parenchyma on that side of the stele adjoining the wound. In the particular example figured the intact superficial cells, and those

immediately subjacent, are occluded by gum. In other cases the thickening is relatively slight, and is accompanied by a local enlargement of the cells (Fig. 5). This type of reaction appears to be common both in the small

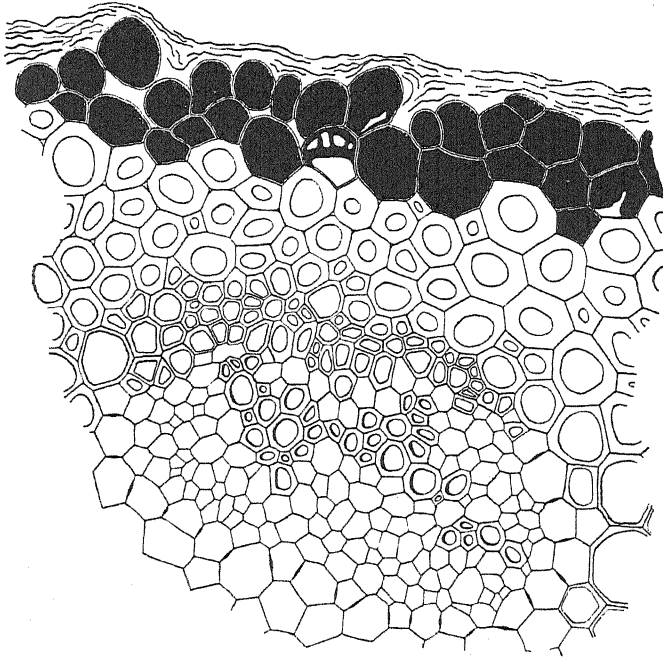


FIG. 4. Transverse section of a small branch with a lateral wound. The scab of dead surface cells is indicated diagrammatically. Below this the intact parenchyma shows an early stage in gummosis. The wound reaction consists of widespread thickening of the walls involving the whole of the cortex, endodermis, and intrastelar parenchyma on the side adjacent to the wound. $\times 250$.

ultimate branchlets and at lower levels. In both slender and more robust branches, however, clearly defined evidences of meristematic activity are not uncommon, and in many cases this leads to the development of a well-marked strip of cambiform tissue below the wound surface. Two cases are figured, one (Fig. 6) in a small twig, and the other in a stouter branch (Fig. 7). The latter is one in which the whole of the outer cortical parenchyma in the neighbourhood of the wound has become involved, and this clearly shows both the initial elongation of the parent cells and their later division to produce a number of meristematic cells. In this particular instance the cells have remained thin-walled, but much more frequently the elongation and division are followed by a thickening of the wall which, as in the examples to which reference has been made previously, is almost entirely cellulosic in the more superficial zone, and which becomes progressively more heavily lignified in the deeper parts. Curiously enough there

often occur small local patches of living meristem actually adjoining the

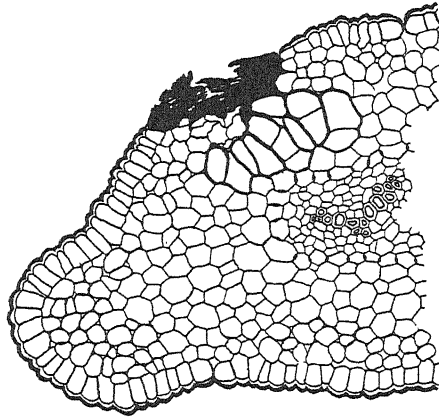
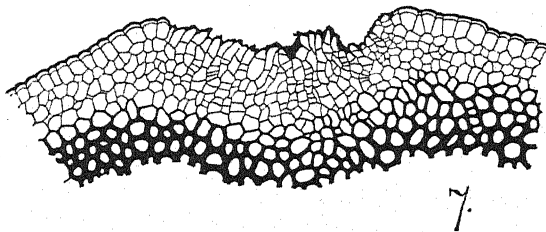
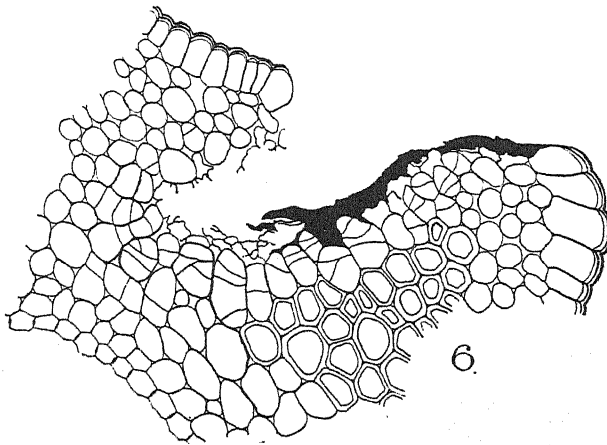


FIG. 5. Transverse section of a small branch with an area consisting of elongated and slightly thickened cells due to wounding. $\times 200$.



FIGS. 6 and 7. Transverse sections of two branches showing the development of unthickened cambiform elements. Fig. 6. $\times 300$; Fig. 7. $\times 150$.

wound surface which retain their thin-walled character. A small patch of this kind flanked on either side by thicker-walled cells is illustrated in

Fig. 8. The whole of the cells figured had cellulose walls, the first indications of lignification occurring at a slightly greater depth.

Although careful search has been made no evidence of superficial suberin deposits has been found. This may be due to the nature of the

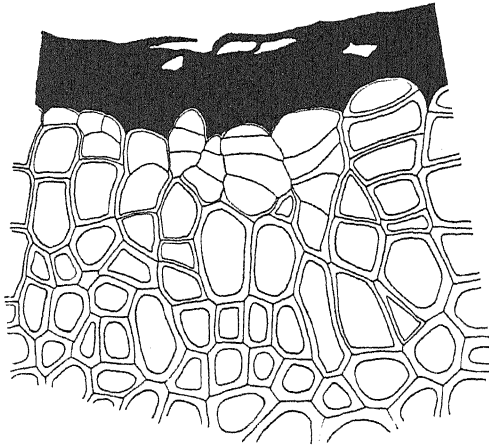


FIG. 8. Small portion of a transverse section showing an advanced stage of gummosis and both thin- and thick-walled cambiform elements. $\times 350$.

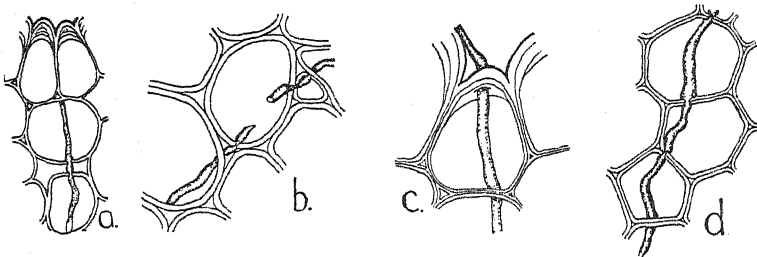


FIG. 9. (a), (b). Fungal hyphae traversing cortical cells and intercellular spaces; (c) fungal hypha penetrating an epidermal cell; (d) fungal hypha traversing three cortical cells and showing constriction at the points of entry and exit. a, b, and d $\times 250$; c $\times 350$.

material, the outer tissues of which are often deeply discoloured, this rendering its detection, if present, difficult. It is suggested that it may be replaced either by the dead remains of the cells at the seat of injury or, where it occurs, by the gummy material resulting from the degeneration of the cells bordering the wound.

In addition to the varying type of wound response exhibited, a number of the specimens examined were infected by the hyphae of a non-septate fungus. These hyphae frequently traverse a large number of cortical cells, showing in many cases the characteristic compression at the points of entry into and exit from each cell (Figs. 9 b, 9 d, 10).

Less commonly, the hyphae penetrate the endodermis and stele, and

a case of this type of infection is shown in Fig. 11, in which endodermis, phloem, and xylem are all involved. No actual example of an infecting hypha was discovered, with the possible exception of that illustrated in,

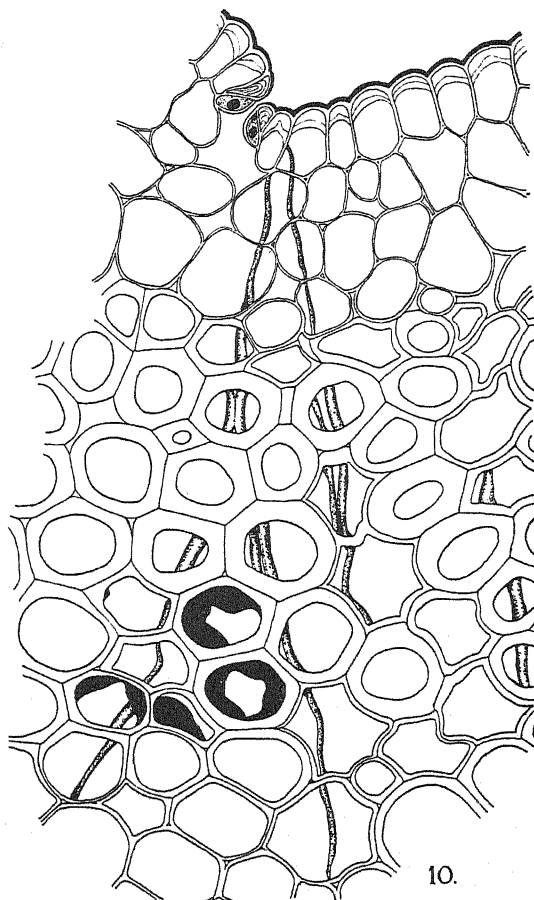


FIG. 10. Fungal hyphae of varying thickness traversing the cortex of a wounded stem.
x 350.

Fig. 9c, in which the hypha is shown both outside the epidermis, and traversing an epidermal and a cortical cell. This might, however, be equally well interpreted as a hypha leaving the tissues.

One feature of some interest shown by the hyphae was the considerable variation in thickness (Fig. 10). This appeared to be due in part at least to the deposit by the cells of the host plant of layers of cellulose upon the external surface of the hypha, this being more marked where the cell walls of the infected cell had undergone thickening. Cells of this type when swollen by treatment by chlor-zinc-iodine showed well-marked bluish violet lamellae, which were also present as a sheath surrounding the enclosed

hyphae, and which in many cases were obviously continuous with the thickening lamellae constituting the modified cell wall (Fig. 12). A thin

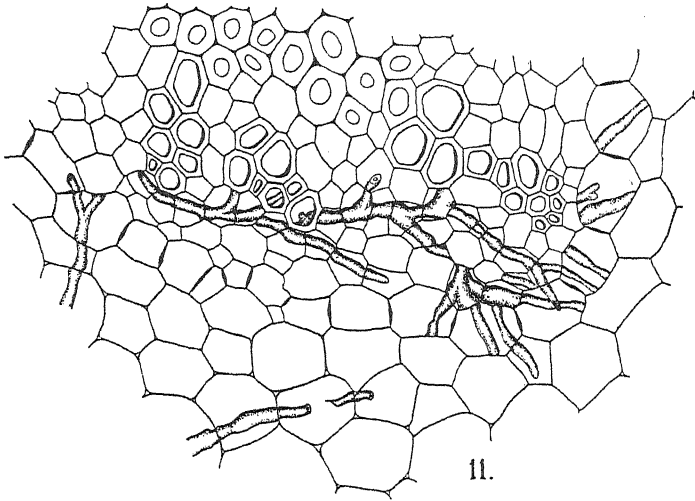


FIG. 11. Similar hyphae in the stele. $\times 350$.

film of lignin was also noted in some cases surrounding the hyphae which were present in the tracheids. These facts suggest that invasion by the fungus occurred at a relatively early stage, and that its hyphae were involved in any subsequent modifications of the walls of the invaded cells. It is possible, of course, that the fungus is identical with that forming the endotrophic mycorrhiza in the subterranean stem since Rayner (9) has shown that such hyphae are more widely distributed in the host plant than has been generally supposed. There is, however, no means of checking this interpretation. Whatever the origin of the hyphae the development of a sheath of cellulose by the cytoplasm of the host must seriously restrict their subsequent activities.

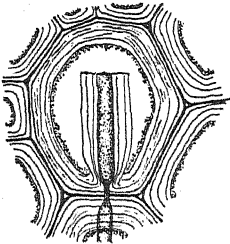


FIG. 12. Appearance of a thickened cortical cell enclosing a fungal hypha, after swelling with chlorzinc-iodine. $\times 700$.

The results obtained in this investigation indicate, as one would anticipate, that the relatively unspecialized tissues respond most actively to traumatic stimulus. The types of reaction ranging from widespread local thickening to the formation of a well-defined local meristem which may or may not be accompanied by subsequent thickening, are similar to those already recorded for the Filicales (4). It is evident that these variations, in *Psilotum*, are not due to the occurrence of an impermeable endodermal barrier whatever may be the case in ferns (6, 7). The endodermis in *Psilotum*, as has already been stated, is of the primary type, and extends

from a little behind the tip of each branch as an unbroken sheath throughout the aerial stem. The leaves play an insignificant part in the assimilatory activities of the plant, their place being taken by the green stem, so that there must be considerable translocation of assimilates across the endodermis throughout its length. It may, of course, be due to moisture variations in the habitat, but this could only be determined by a series of experiments with living plants.

SUMMARY.

1. Aerial stems of *Psilotum triquetrum* showing injury may be grouped under two heads, namely:

(a) Those in which the injury involves the apex of the branch.

(b) Those with lateral wounds.

2. In both types of wound the intact cells near the seat of injury have their walls discoloured owing to the deposit of tannins.

3. In the first type the injured apex is covered by a cap of dead cells.

4. Below this a series of radiating fissures extends downwards into the cortex and stele. These are filled with gum, and are surrounded by cells, showing varying degrees of meristematic activity.

5. Wounding also results in the development of local thickenings of the cell walls of both the intrastelar and extrastelar parenchyma, these thickenings being deep brown in colour owing to the presence of phlobaphenes.

6. Apical injury causes degeneration of the stelal tissues for a greater distance downwards than is the case with the cortical tissues. The tracheids are occluded by gum, and below this level show a somewhat anomalous distribution.

7. In the case of lateral injuries the subjacent cells may either undergo thickening or become meristematic or both.

8. The meristematic activity ranges from cell-elongation to the production of typical cambiform elements.

9. Where thickening occurs the thickening substance is cellulose in the outermost cells and lignin in those which are deeper seated.

10. In one batch of material examined wounding was accompanied by extensive local gummosis. This resulted from the conversion of the cellulose of the cell walls into gummy matter, and frequently led to the partial or complete coalescence of the cells.

11. No suberin deposits were detected at the surface of wounds.

12. In some cases considerable numbers of fungal hyphae were present in the tissues. These were encased in cellulose lamellae deposited by the host plant, and it is suggested that this would render them incapable of causing further injury.

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Observations on the Anatomy of Teratological Seedlings.

VII. The Anatomy of some Polycotylous Seedlings of *Impatiens Roylei*, Walp.

BY

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AND

A. EVELYN WOOD, M.Sc.

With thirty Figures in the Text.

THE seedling of *Impatiens Roylei*, like that of *Sinapis alba* (2), shows deviation from the normal dicotylous condition in the direction both of syncotyly and of polycotyly. The syncotylous modification, which is the form of abnormality of most frequent occurrence in this species, was subjected to detailed study by Holden (4), and Holden and Daniels (6), and in the course of their investigation two polycotylous specimens were found and described (6).

Since these showed rather peculiar transition phenomena it seemed desirable to carry out a fuller investigation of this type of modification, but the work, of which the present paper is the outcome, has been much delayed owing to the rarity of the polycotylous condition in this species. Not only is the percentage of polycotylous seedlings extremely low, but a very thorough search has resulted in the discovery of hemitricotylous and tri-cotylous types only, whereas in other plants producing polycotylous seedlings the full range of types from the hemitricotylous to the tetracotylous form has been described (1, 5). The material available has, however, provided further examples of peculiar transition which seem to merit some brief description.

The structure of the dicotylous seedling of *Impatiens Roylei* has been described in some detail in an earlier paper of this series (4), so that only a very brief outline of the seedling structure and the transition phenomena need be given here. At the base of the cotyledon petiole the vascular system consists usually of three strands, namely, a midrib strand composed of a xylem group with mesarch or exarch protoxylem and two laterally

placed phloem groups, and two lateral strands. The six strands derived from the two cotyledons enter the hypocotyl where each midrib strand becomes connected with a root-pole, whilst the laterals fuse in pairs to form two other poles, so that a tetrarch condition obtains in the major portion of the hypocotyl and in the root. At the stage at which the seedlings are usually examined the leaf whorls at the first and second nodes of the epicotyledonary axis are generally present in an early stage of development, and in the normal seedling there are two members in each whorl. A characteristic feature of the seedling is the development of a whorl of four lateral roots at the junction of the hypocotyl and the main root.

Hemitricotylous seedlings.

The thirteen hemitricotylous seedlings examined showed the usual variation in form of the abnormal cotyledon, ranging from a very slight lobing at the apex to a definite median cleft, which in some cases involved the upper portion only of the lamina while in others it extended almost throughout the cotyledon (Figs. 1 and 2). In cases of very pronounced fission each lobe of the cotyledon was of course supplied by a midrib bundle, but in seedlings with only a slight lobing the venation did not correspond at all closely with the degree of lobing, so that while in some seedlings an apparently simple midrib was present, in others the cotyledon lamina showed two well-marked median veins (Figs. 3 and 4), these either fusing at a lower level or remaining distinct throughout the cotyledon.

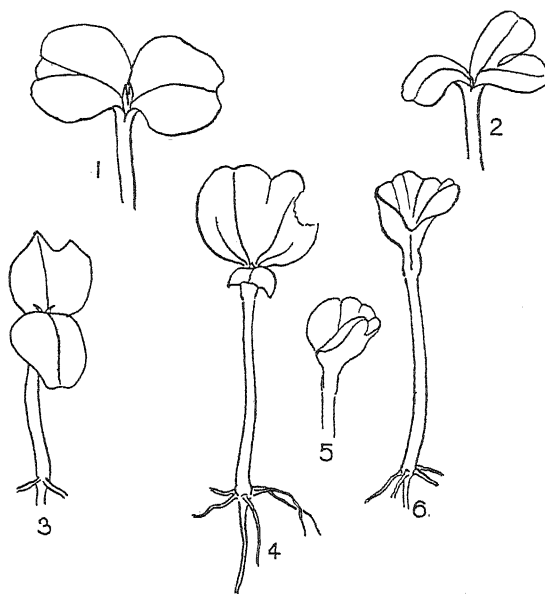
Three of the seedlings which showed only a very slight lobing of the abnormal cotyledon were quite normal as regards the vascular structure in the cotyledon, and the behaviour of the strands in transition was exactly like that observed in the dicotylous seedlings as described above.

Of the seedlings with a more distinctly bifurcated cotyledon three showed a fairly simple anatomical structure, since the two lobes of the cotyledon were supplied by midrib bundles of collateral structure which approached one another in the lower portion of the cotyledon and finally united to form a single bundle. This behaved in transition like the midrib of a normal cotyledon, so that tetrarchy obtained in the hypocotyl and root. The level of union of the bundles varied in the different seedlings, and it is noteworthy that in one case, although the abnormal cotyledon showed only a slight apical cleft, the two strands remained distinct until the base of the petiole was reached.¹

In two other seedlings (A and B), which were of the same general type in that they showed tetrarch structure throughout the hypocotyl, the behaviour of the midrib strands was different from that just described. Seedling A was nearly tricotylous since the cleft in the abnormal cotyledon extended throughout the lamina and the upper portion of the petiole. The three

¹ This type of structure has been termed 'Type α ' in earlier papers of this series.

laminae were equal in size and each had the venation characteristic of the species, with a midrib and inner and outer lateral strands. In the lower undivided region of the petiole the inner lateral strands fused with their



FIGS. 1-6. 1-4. Sketches of seedlings illustrating variations in depth of lobing and behaviour of midrib bundles. 5-6. Drawings of amphitrisyncotylous seedling.

respective midribs, and the latter became closely approximated. One of the midrib strands now showed bifurcation of the phloem and a change in the position of the protoxylem, whilst the other bundle retained its collateral structure, and after swinging through an angle of 90° joined the other strand which at this level showed exarch protoxylem. From this point onwards transition followed the normal course and tetrarchy was established.

Seedling B was similar, except that the cotyledon was not so deeply lobed, no well marked inner lateral strands were present, and the union of the two median bundles took place at a higher level.

In all the remaining hemitricotyls the principal strand supplying each lobe of the abnormal cotyledon behaved like an ordinary midrib bundle entering the hypocotyledonary axis independently and there undergoing 'rotation' to produce an exarch xylem group.¹ A third pole was formed by the midrib of the normal cotyledon, while the four laterals of the two cotyledons fused in pairs to form two other poles so that a pentarch structure obtained in the axis just below the cotyledonary node. The subsequent behaviour of the xylem groups varied however in different seedlings. In one case the two poles produced by the median strands of the

¹ Referred to as 'Type *b*' in earlier papers.

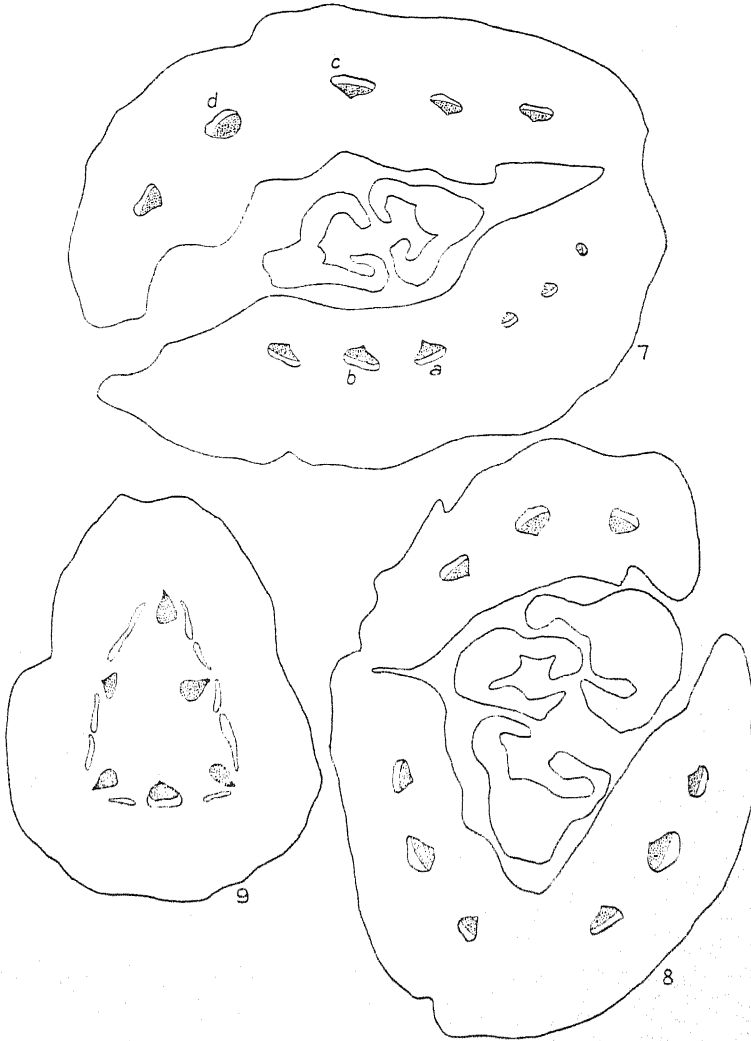
lobed cotyledon approached one another at a fairly high level in the hypocotyl, and united to form a single pole so that tetrarchy resulted. In a second seedling the two poles became very closely approximated at the upper end of the hypocotyl, but maintained these positions for some considerable distance although eventually fusion took place with the production of normal tetrarchy. Finally, in two other cases the pentarch structure prevailed throughout. It is noteworthy that in the seedlings showing reduction from pentarch to tetrarch structure the cleft in the cotyledon was quite deep, but those showing persistent pentarchy had a less deeply divided cotyledon.

In another hemitricotyl the structure was somewhat different from that of the seedlings described hitherto. The seedling appeared to be a hemitricotyl in which the two midribs of the lobed cotyledon were well marked, but microscopic examination showed that in the superficially normal cotyledon also the vascular structures were rather abnormal, since apparently two midribs and two groups of lateral strands were present just as in the lobed cotyledon (Fig. 7). The two midribs of the lobed cotyledon resembled those of hemitricotyls already described, since they entered the hypocotyl independently and gave rise to two root-poles. One of the median strands of the other cotyledon formed a third pole, but the other swung across towards the outer lateral, and after giving off a small bundle which subsequently died out, it joined the lateral strand to form one large bundle. This bundle together with the adjacent lateral strand of the lobed cotyledon formed a fourth pole, and the two lateral strands on the opposite side of the axis united to produce a fifth pole. This decreased in size until it consisted of a single xylem element only, which was however persistent throughout the seedling.

In all the hemitricotyls described hitherto the epicotyl was of normal structure, two leaves being present at the first node. In hemitricotyl C there was a more marked tendency towards trimerous symmetry, this being shown not only in the presence of three leaves at the first epicotyledonary node, but also in the number and behaviour of the vascular strands in the abnormal cotyledon. The bifurcation of the cotyledon extended for more than half the length of the lamina, and each lobe was supplied by a midrib and both inner and outer laterals (Fig. 8). On entering the hypocotyl the three midribs formed three exarch xylem groups, and the two inner laterals of the lobed cotyledon united to form a collateral bundle which persisted for some distance in the hypocotyl. The two remaining pairs of laterals fused and formed xylem poles, so that for a time the hypocotyl possessed five alternating groups of xylem and phloem and one collateral bundle (Fig. 9). On the disappearance of the latter simple pentarchy prevailed for some distance, this ultimately being replaced by tetrachy owing to the dying out of one xylem pole formed by lateral strands.

Tricotylous seedlings.

In the tricotylous seedlings examined the structure most commonly occurring in the hypocotyl was pentarchy, but since the method by which



FIGS. 7-9. 7. Diagram of hemitricotyl showing two median strands (*a* and *b*) in externally normal cotyledon; *c* and *d* midrib bundles of lobed cotyledon. 8-9. Diagrams of hemitricotyl C showing well developed inner lateral strands.

this was attained varied somewhat a brief description of each seedling is necessary.

In tricotyl A each cotyledon petiole showed the normal bundle arrangement, that is, a midrib bundle and two laterals. Two of the

cotyledons fused together slightly above the cotyledonary node and the adjacent lateral strands united so that eight bundles entered the hypocotyl, namely, three midrib strands, four lateral strands, and one collateral strand composed of two fused laterals. The four lateral strands quickly united in pairs with subsequent production of an exarch xylem group in each case, and each of the three midrib strands also formed a pole, so that five xylem poles were organized. The collateral strand previously mentioned died out very quickly after entering the hypocotyl, but for some time the arrangement of the remaining xylem and phloem groups showed distinct asymmetry.

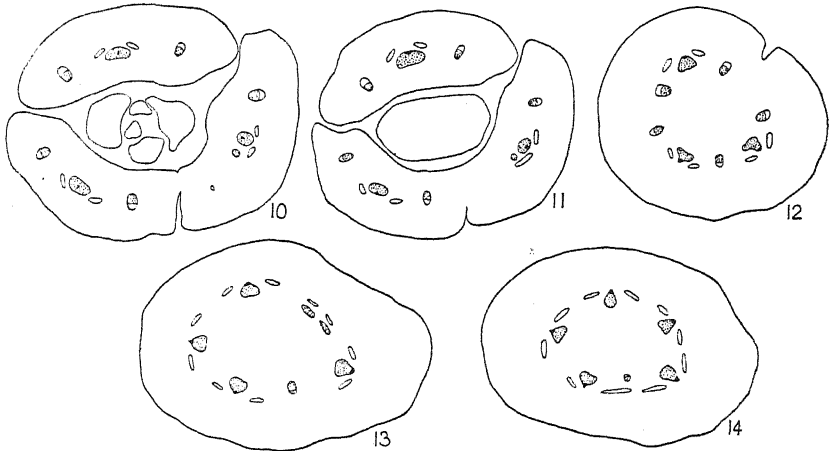
In tricotyl B the behaviour of the strands was very similar. Two of the cotyledons underwent slightly premature fusion, but all nine strands entered the hypocotyl separately. The adjacent laterals of the two cotyledons showing premature fusion, after uniting to form a collateral bundle, quickly died out without any attempt at the production of an exarch structure, so that pentarchy was established in the upper portion of the hypocotyl and extended throughout the seedling.

Tricotyl C was of a slightly different type since, although each cotyledon petiole had the characteristic three bundles, one lateral strand became much reduced near the base and finally fused with the midrib strand so that only eight bundles entered the axis, one lateral strand lacking a fellow strand from the adjacent cotyledon. This unpaired lateral persisted for some distance in the hypocotyl as a collateral strand, but finally disappeared before root level was reached. In this seedling also there was slightly precocious fusion of two of the cotyledons (Figs. 10–14).

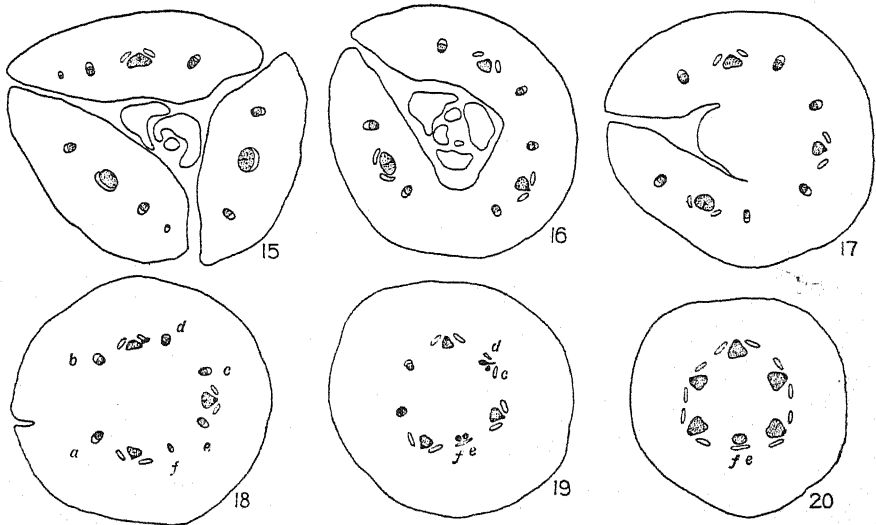
It may be noted that in the seedlings described the epicotyl was normal in form, and that there was no epicotyledonary leaf alternating with the two cotyledons which united at a level slightly above the node (Fig. 10).

The structure of the two seedlings tricotyl D and tricotyl E showed that the early fusion of the two cotyledons had no close connexion with the failure of the lateral strands to form a root-pole. In tricotyl D the three cotyledons fused to form a U-shaped structure (Fig. 16), and fusion with the axis took place before the final union of the cotyledons was accomplished. Of the nine bundles which entered the hypocotyl the three midrib strands formed poles in the usual manner, while two other poles were connected with the laterals *a* and *b*, and *c* and *d*, respectively. The remaining lateral strands *e* and *f* fused to form a collateral bundle (Figs. 19, 20) which, although persisting for some little distance, ultimately died out so that pentarch structure prevailed in the hypocotyl. Although the laterals *c* and *d* united to form a pole, bundle *d* was much larger than *c* and played the major part in pole organization (Fig. 19). In this seedling there were three leaves in the first epicotyledonary whorl (Fig. 16).

In tricotyl E there was no sign of early fusion of cotyledons, but in one cotyledon the lateral strands approached the midrib so that at the base of



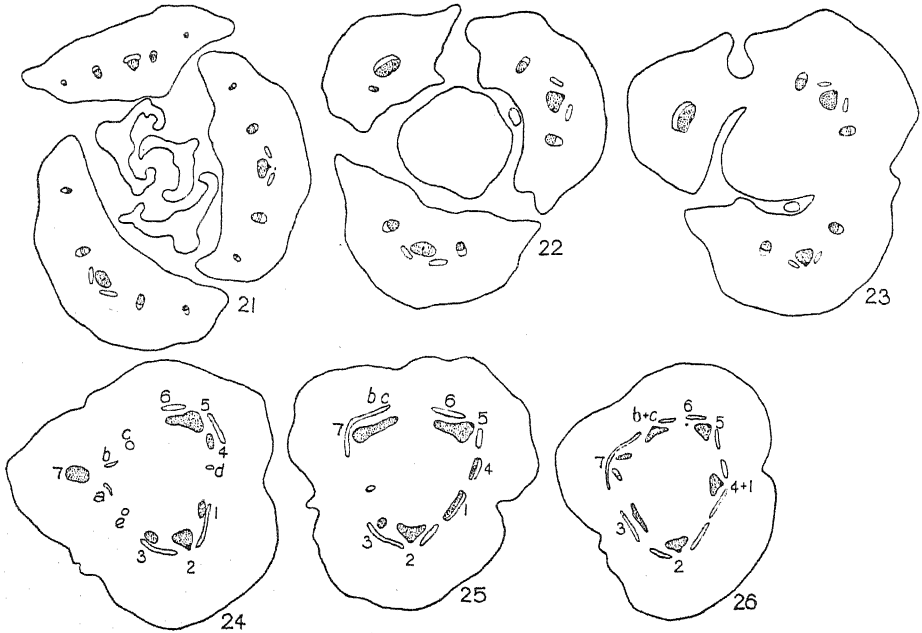
FIGS. 10-14. Diagrams of tricotyl C showing peculiar behaviour of lateral strands.



FIGS. 15-20. Stages in transition of tricotyl D.

the petiole only one rather flattened bundle was present. In the hypocotyl each of the three midrib bundles formed a root-pole, and one pair of laterals produced a fourth pole. On one side of the midrib of the abnormal cotyledon a root-pole associated with lateral strands was organized, whilst on the other side appeared a collateral strand. The hypocotyl thus contained five exarch xylem groups with alternating phloem groups, and a collateral strand which persisted almost throughout the length of the axis. The seedling was rather an old one, and it was difficult to determine exactly what part was

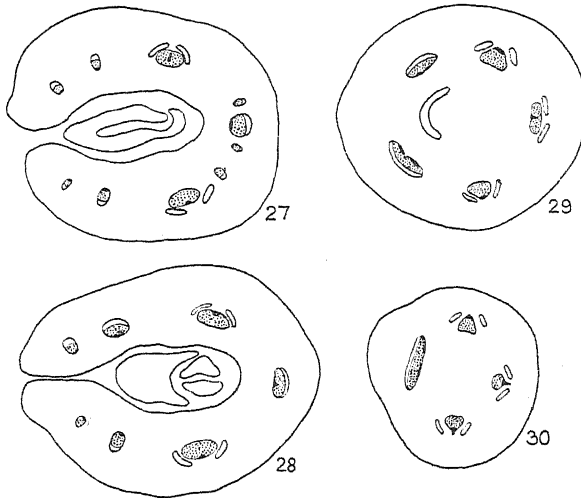
played by the vascular tissues of the abnormal cotyledon, but it apparently contributed a few elements to both the xylem pole and the collateral strand, these being mainly formed by the lateral strands of the other two cotyledons.



FIGS. 21-6. Stages in transition in tricotyl F.

Tricotyl F was a seedling displaying most unusual structure. The epicotyl was trimerous, two whorls of leaves being developed. Each cotyledon had the normal number of vascular strands, but these were smaller in size in one cotyledon than in the others, and at the base of the petiole these smaller bundles united to form one strand while the other two petioles had the normal three-bundle structure (Fig. 22). The cotyledon petiole with the single bundle was the last to fuse with the axis, and its vascular strand was also late in joining the hypocotyledonary stele (Figs. 23 and 24). A short distance below the cotyledonary node the structure illustrated in Fig. 24 was found. Strands 1, 2, and 3 were the three strands from one normal cotyledon, strands 4, 5, and 6 were derived from the second cotyledon, while strand 7 was the sole contribution from the peculiar cotyledon. Bundles *a*, *b*, *c*, *d*, and *e* were epicotyledonary strands. The two midrib strands 2 and 5 formed two root-poles, the adjacent lateral strands 1 and 4 formed a third pole, while the bundle of the abnormal cotyledon curved very sharply to enter the vascular ring so that it was cut almost longitudinally in the transverse section of the axis, and became transitorily connected with the epicotyledonary strands *b* and *c* (Fig. 25).

Separation of the strands followed quite soon, after which the xylem group composed mainly of *b* and *c* became exarch, and a break occurred in the phloem ring immediately outside (Fig. 26). Bundle 6, a lateral strand which should take part in pole formation in this region of the axis, moved



FIGS. 27-30. Stages in transition of amphitrisyncotyl H.

across towards group *b*, *c*, but disappeared without becoming associated with the xylem pole. The xylem of the collateral bundle 7 now bifurcated and the half strands moved off to left and right, leaving a small phloem group *in situ*. One of the xylem groups joined the pole formed by bundles *b* and *c* together with the strand previously derived from bundle 7; the other xylem group joined the lateral strand 3, which in conjunction with the epicotyledonary bundle *e* was forming a fifth root-pole. The pentarch structure so produced obtained throughout the seedling. It may be noted that a small bud appeared in the axil of each normal cotyledon, but not in that of the abnormal cotyledon.

Tricotyl G was the only specimen examined in which all the lateral strands behaved as in the normal dicotylous seedling, fusing in pairs to form three poles so that hexarchy prevailed in the hypocotyl. Unfortunately the root had been injured so that it is impossible to say whether the hexarch condition persisted throughout the seedling.

Seedling H stood apart from all the other tricotyls both in external form and in the transition phenomena. It was an amphitrisyncotyl with the cotyledons fused for the greater part of their length, except at one side where the fusion extended for a short distance only. A section through the cotyledon, therefore, showed a U-shaped structure in which three prominent vascular bundles represented midrib strands, while the lateral strands with the exception of the outer ones were very insignificant. At a

somewhat lower level the cotyledons assumed the form of a cylinder surrounding the epicotyledonary leaves which showed deviation from the normal condition since there was only one member in the first leaf whorl, this appearing on the side where the fusion of the cotyledons was least pronounced (Fig. 27). The hypocotyl possessed only three exarch xylem groups, these being connected with the three midrib strands. The outer laterals fused to form a large bundle, which did not, however, show definite pole organization but remained collateral in structure. The other lateral strands disappeared above the cotyledonary node (Fig. 28).

DISCUSSION.

In spite of the comparatively small number of seedlings examined it is evident that the polycotylous material of *Impatiens Roylei* presents for the most part a range of structure similar to that found in other species producing polycotyls, since in some seedlings each of the median strands of two cotyledons or cotyledon lobes forms a root-pole, while in other cases the strands of two cotyledon lobes contribute equally to the formation of a single root-pole, or the bundle of one lobe behaves like a lateral, and plays only a minor part in pole organization (cf. *Althaea rosea* (1)).

In seedlings of the *Impatiens*-type with tetrarch symmetry in the normal seedling due to participation of the lateral strands in root-pole formation, the polycotyls show a wider range of structure than those of the normally diarch types, because of the variations in development of the lateral strands and their failure in some cases to take part in root-pole organization. In the hemitricotylous seedlings examined the lobed cotyledon develops outer lateral strands as usual, but inner lateral strands are usually either absent or very feebly developed, and appear in the upper region only of the cotyledon. Such seedlings have tetrarch or pentarch structure according to the behaviour of the two median strands of the lobed cotyledon. In one hemitricotyl and in the tricotyls all the lateral strands are developed, but only in one instance are all the possible xylem poles organized, this resulting in hexarch structure. In all other cases two of the lateral strands undergo fusion to form a collateral strand, which may either disappear quickly or persist to quite low levels in the seedling. The tricotylous specimen of *Impatiens* described in an earlier paper (6) also exhibited a collateral strand formed by laterals, but this seedling was peculiar in several respects. Similar failure of the lateral strands to form a root-pole was found in some of the syncotylous *Impatiens* seedlings (4), and in these seedlings it could be correlated with obvious diminution in importance of the strands in the cotyledon. In some of the tricotyls also the laterals seem somewhat small at the base of the cotyledon, but this is not so in all cases, and it seems difficult to explain why certain lateral strands should be of diminished

importance in cotyledons equal to the others in size and showing symmetrical development.

Of the species producing polycotyls, which have been studied in detail, *Althaea rosea* (1) offers most points for comparison with *Impatiens*, since both have tetrarch symmetry in the normal seedling, whereas the majority of these types have diarch structure. The dicotylous specimens differ, however, since in *Althaea* the lateral strands are not independent throughout, but fuse with the midrib at the base of the cotyledon petiole. They subsequently take part in pole formation however, and indeed, each lateral strand may form a pole independently, so that the hypocotyl and root of the dicotylous seedling may exhibit hexarchy. In view of this it is interesting to note that in some of the tricotylous seedlings of *Althaea* some of the lateral strands failed to appear in the hypocotyl. When they were present, however, they always contributed to root-pole formation. This was not the case in one tetracotylous seedling, where a lateral strand persisted as a collateral bundle in the hypocotyl and finally died out.

This behaviour of the laterals in both *Althaea* and *Impatiens*, and also the fact that the median strand of a cotyledon or well marked cotyledon lobe can act as an ordinary lateral strand in the hypocotyl, offers additional evidence of the inadequacy of the theory which would explain variations in transition phenomena solely on the grounds of changes in functional importance of the strands in the cotyledons.

Seedling H, the amphitrisyncotyl, does not show quite such close union of its members as did the seedling of the same type previously described (6), and the modification of internal structure is similarly less marked, since the laterals on one side are well developed, although they do not form a definite pole. In this respect they agree with other amphisyncotylous seedlings, such as seedling C (6).

The transition phenomena in tricotyl F call for some comment, since the bifurcation of the cotyledonary bundle is a somewhat unusual phenomenon. Seedling G, described in a previous paper (6), offers some points of similarity, but in that instance there was no clearly marked bifurcation of the strand, and a small amount of metaxylem was left in the cotyledonary plane. Moreover, the branches given off from the peculiar cotyledon bundle united with the adjacent laterals to form poles, whereas in tricotyl F of the present series an epicotyledonary strand seems to play a relatively important part in the formation of one root-pole, and the lateral cotyledonary strand dies out.

A dicotylous specimen of *Impatiens* has been described (4), in which one cotyledon was much smaller than the other and possessed apparently no midrib, since the collateral strands present in the cotyledon petiole massed together to form two lateral groups which ultimately united with the adjacent lateral groups, so leaving the cotyledonary plane quite devoid

of vascular tissue. In the seedling under discussion, however, the cotyledonary strands at first form one median bundle, such as occurs in tricotyl E where it proceeds to form a root-pole in the usual manner.

With regard to other species, Miss Davey (3) reports that in *Pterocarya rhoifolia* the cotyledon petiole contains a median protoxylem and two lateral strands on each side. At the base of the petiole each set of strand fuses to form one bundle, and the median group bifurcates, each half joining a lateral group so that two fairly widely separated endarch bundles enter the hypocotyl from each cotyledon. At a lower level a protoxylem group reappears in the cotyledonary plane, and a pole is organized in the normal manner. This temporary lateral concentration of the vascular tissues in the cotyledon, followed by normal procedure in the hypocotyl, seems quite distinct from the structure found in *Impatiens* seedling F.

The behaviour of the peculiar cotyledonary strand in tricotyl F seems to have no connexion with the phenomena usually associated with schizocotyl and polycotyl, and it can only be regarded as an aberrant vascular structure which might equally well occur in a dicotylous specimen (where aberrant structures, as already indicated, are not unknown), but for which there is at present no explanation.

SUMMARY.

1. The seedlings of *Impatiens Roylei* examined consist of hemitricotyls, showing all degrees of lobing, tricotyls, and an amphitrisyncotyl.
 2. In some hemitricotyls the modification of the vascular system is confined to the cotyledon, the hypocotyl and root showing the tetrarch symmetry characteristic of the normal seedling. In such cases the vascular strands supplying the lobes of the abnormal cotyledon behave either as halves of the ordinary midrib bundle or as entire midrib and enlarged lateral.
 3. In the other hemitricotyls the abnormal cotyledon is associated with two root-poles, and pentarchy obtains at least in the upper part of the hypocotyl.
 4. In the tricotylous seedlings hexarchy is rare; pentarchy is the typical condition, but may be attained in various ways.
 5. The structure of these seedlings is compared with that of *Althaea rosea* and other seedlings showing a similar type of transition.
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The Phylogeny of Monocotyledons

BY

D. H. CAMPBELL.

With eleven Figures in the Text.

ONE hundred and forty years ago Jussieu proposed the terms Monocotyledons and Dicotyledons to distinguish the major divisions of the flowering plants; and except that the Gymnosperms are now excluded from the Dicotyledons, this classification has been maintained, the differences in the embryos of the two divisions being held of fundamental importance. Jussieu's further division of the Dicotyledons into Apetalae, Polypetalae, and Monopetalae, except for a change in terminology and the inclusion of the Apetalae with the Choripetalae, is still in general use in our manuals.

Compared with the sweeping changes in the taxonomy of the Animal Kingdom, it must be confessed that the botanists—to put it mildly—have been somewhat conservative.

While no botanist can fail to recognize the great importance of Jussieu's contributions, and the essential correctness of his conclusions, as based upon the data available to him, it is evident that in view of our greatly increased knowledge, both in the vastly larger number of known species and detailed studies of structure and development, a very complete overhauling of the fundamental problems dealing with the interrelationships of the Angiosperms is urgently needed.

In a recent paper (14) the writer has reviewed some of the latest conclusions as to the phylogeny of the Angiosperms, and in the present communication it is proposed to deal specially with a single topic—the much discussed question of the systematic position of the Monocotyledons.

While admitting the unmistakable relationships between all existing Angiosperms, it is becoming more and more evident that the arbitrary division into two co-ordinate sub-classes, Monocotyledons and Dicotyledons, is not an entirely natural one. That there have been more than two main lines of evolution from some older stock which no longer exists seems certain, and it is much more likely that instead of two primary divisions, or sub-classes, we shall have to recognize a much greater number, and these sub-classes, or phyla, must be based upon something more than the superficial characters which hitherto often have been deemed sufficient.

A critical examination of the differences commonly accepted as distinguishing the sub-classes will clearly show that none of them is really adequate. We may point to 'typical' flowers like a lily or geranium, for instance, as illustrating the floral characters of the two sub-classes; but if instead we should select the flowers of *Sparganium* and *Populus*, how are we to say which is Monocotyledon and which Dicotyledon? The explanation that the latter are reduced from the 'typical' flower is not very convincing—especially as it happens that these are among the oldest known Angiosperms.

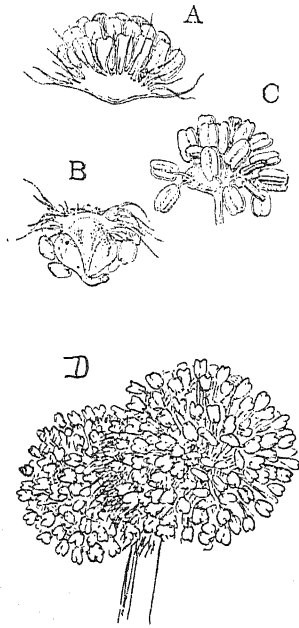


FIG. 1. A, B, C Staminate flowers of *Populus trichocarpa*. D. Staminate flowers of *Sparganium eurycarpum*.

So also the vegetative structures. The herbaceous stem, with its scattered 'closed' bundles, characteristic of most Monocotyledons, is practically duplicated by a considerable number of Dicotyledons, e. g. *Peperomia*, *Podophyllum*; and the broad reticulate veined leaf, found in so many Dicotyledons, occurs in such Monocotyledons as *Trillium* and many Araceae.

Even the embryos are not without exceptions. Some Dicotyledons—e. g. *Abronia*, *Cyclamen*, *Ranunculus*, *Ficaria*—are 'Pseudo-Monocotyledons', and certain Monocotyledons, like *Zannichellia* and *Dioscorea*, have a terminal stem apex, as in Dicotyledons, and the

cotyledon is a lateral structure.

In short, it is clear that we can find no satisfactory criteria which indicate such fundamental differences as are implied in the recognition of the Monocotyledons and Dicotyledons as sub-classes.

While it is true that the great majority of Monocotyledons are remarkably uniform in their general morphology, as well as in their anatomy and their floral structures, there are certain families, e. g. Pandanaceae, Araceae, Naiadaceae, which are very different, especially in their floral structures, and which are difficult to homologize with 'typical' monocotyledonous flowers. In most speculations concerning them, these aberrant floral types are either entirely ignored or given scant consideration, being regarded as 'reduced' from the typical floral structures.

Naturally there has been much speculation as to the relationships existing between Monocotyledons and Dicotyledons. The *Helobiales*, which include several families of the simpler Monocotyledons, show some striking likeness to the *Ranales*, and suggest a possible real relationship between the two orders. The prevalent view is that the Monocotyledons have originated

from ancestors related to the living *Ranales*, and hence must be regarded as a younger group than the Dicotyledons.

Strasburger (46), from a comparison of the *Gnetales* and Dicotyledons, concluded that the latter represent three independent phyla derived from the *Gnetales*, and that from one of these the Monocotyledons subsequently branched off. It is probable that Strasburger's view has strongly influenced the present theories as to the derivative character of the Monocotyledons.

Somewhat later, Kny (30) decided that the Monocotyledons were developed independently from Filicineae, and still later Solms-Laubach (45) came to the conclusion that the two groups of Angiosperms arose independently from Gymnosperms.

The view that the Monocotyledons probably represent a single phylum derived from the Dicotyledons is indicated in some of the more recent work on the taxonomy of the Angiosperms. Hutchinson (27) believes that the Angiosperms are descended from some common ancestor related to the fossil *Bennettitales*, and that the nearest existing relations of the latter are to be found in the *Magnoliales* and *Ranales*, which he separates as independent orders. From the *Ranales* he would derive the Monocotyledons.

Hutchinson evidently has been strongly influenced by the work of Arber and Parkinson (2), who have elaborated the theory of the origin of the Dicotyledons from forms related to the *Bennettitales*. They hold that the primitive Angiosperm flower was similar to that of *Magnolia* or *Ranunculus*, and from flowers of this type all floral types, both of Monocotyledons and Dicotyledons, have been derived.

Miss Ethel Sargent (41), in a very careful and thoughtful paper, came to a somewhat similar conclusion as to the flowers of the hypothetical primitive Angiosperms, and also decided that these were Dicotyledons from which later the Monocotyledons were derived.

Wettstein (47), while believing that the Dicotyledons represent several independent phyla derived from Gymnosperms, probably related to the *Gnetales*, considers also that the Monocotyledons originated from the dicotyledonous Polycarpicae.

Perhaps the most important recent contribution to the subject is Engler's introduction to the Angiosperms, in the last edition of the 'Natürlichen Pflanzenfamilien' (19).

Engler finds neither the Arber theory of the origin of Angiosperms from *Bennettitales* nor Wettstein's derivation from *Gnetales* satisfactory, and feels compelled to propose an hypothetical ancestral group 'Protangiosperms', which have much in common with Miss Sargent's Primitive Angiosperms, but differ in certain particulars. He assumes that from the protangiosperm stock many independent phyla originated, some Monocotyledons, others Dicotyledons, i. e., that neither has given rise to the other.

However, if the assumption is made that this is not the case, he thinks it more likely that the Dicotyledons are the older type.

The Protangiosperms are assumed to have been bisporangiate, apetalous, or with a rudimentary perianth, and anemophilous; stamens and carpels indefinite in number, the latter usually free, but possibly united in some cases. The Protangiosperms included both Monocotyledons and Dicotyledons, and there were both herbaceous and woody types. The embryo sac is assumed to have been 8-nucleate, as in the majority of living Angiosperms.

These Protangiosperms were widespread during the middle Mesozoic, and from them numerous phyla of true Angiosperms developed. Engler believes that no great division of existing Angiosperms has been derived from any other one, but the main living phyla are parallel developments, and therefore it is not necessary to assume that the Monocotyledons have been derived from Dicotyledons, but may have come directly from monocotyledonous Protangiosperms. Of the existing Angiosperms, Engler thinks the *Piperales* and *Pandanales* most resemble his hypothetical Protangiosperms.

The absence of fossil Protangiosperms Engler explains by assuming that many of them were herbaceous, and not likely to leave recognizable fossils, while the tissues of the woody species would not be clearly distinguishable from those of true Angiosperms.

The possibility of a derivation of Angiosperms from fern-like ancestors is also considered by Engler, who calls attention to the similarities in the vascular structure of some of the Eusporangiate Ferns, especially the Ophioglossaceae, and the Angiosperms. In this case, as Kny holds, the Monocotyledons, especially in their embryos, would come nearer to the Ferns. The marked resemblances in the embryo, as well as the tissues, between *Isoetes* and the simpler Monocotyledons, has been cited by Coulter (16) as perhaps indicating a real relationship.

The Flower.

While the great majority of Monocotyledons have flowers of the familiar type, i. e. with all the floral organs in threes, this usually is not the case in some of the lower orders where the flowers are often diclinous, and vary greatly in the number of floral parts. The flower may consist of a single stamen or carpel, and in *Naias* (Fig. 2) these are remarkably alike in their origin and structure. Both stamen and carpel are formed from the apex of a shoot, and both macrosporangium and microsporangium are provided with integuments. It is difficult to understand how such flowers could have been reduced from the ordinary monocotyledonous type; and whether or not these extremely simple floral structures are to be considered

as primitive, they can hardly be homologized with any of the petaloidous forms.

Diclinous flowers are characteristic of certain families, e.g. Pandanaceae, Typhaceae, Hydrocharitaceae, and are common in others, like the Potomo-

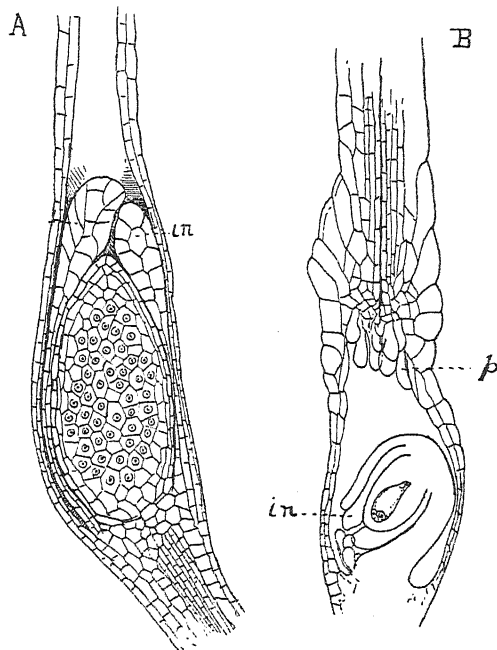


FIG. 2. A. Staminate. B. Pistillate flowers of *Naia flexilis*. in = integument.

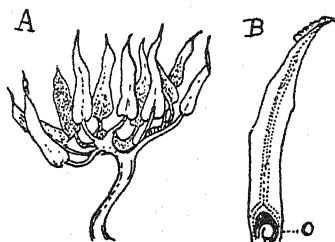


FIG. 3. A. Staminate flower of *Pandanus Lais*, Kurz. B. Pistillate flower of *P. Artocarpus*, Kurz. A. After Solms-Laubach.

getonaceae, Araceae, Cyperaceae, Palmaceae. In *Pandanus*, which Engler points out as the nearest approach to his Protangiosperms, the pistillate flower consists either of a single carpel, or of several more or less closely united. The staminate flower has an indefinite number of stamens (Fig. 3). In most of these diclinous flowers there is either a complete absence of any perianth, or this is composed of inconspicuous scale-like leaves.

The Palms and Araceae offer many examples of extremely simple

unisporangiate flowers, but these families also include genera with flowers conforming to the usual monocotyledonous type, and the diclinous forms might legitimately be interpreted as reduced from bisporangiate ones.

The diclinous flowers characteristic of so many of these lower Monocotyledons might very well, so far as their structure is concerned, be compared with the similar flowers of the lower Dicotyledons, such as the Casuarinaceae, Juglandaceae, Piperaceae, &c., whose relation to the petaloideous Dicotyledons is extremely problematical.

Both Engler and Wettstein think it quite likely that the diclinous condition was developed in some of the ancestors of the Angiosperms, and thus may have been passed on to several quite independent phyla, both of Monocotyledons and Dicotyledons, which would have no direct relationship with those phyla in which bisporangiate flowers are the rule.

In view of the fact that the living representatives of some of the oldest known Angiosperm fossils, e.g. *Populus*, *Sparganium*, are normally diclinous, this condition as it now occurs in so many of the lower orders of both Monocotyledons and Dicotyledons would make this view more plausible than one which believes that all these diclinous flowers are the result of reduction from bisporangiate petaloideous ancestors.

The similarities in the flowers of the Alismaceae and those of the Ranunculaceae have often been emphasized, and taken as indicating a possible relationship between the two families. Attention has also been called to the monocotyledonous characters of the Nymphaeaceae and some of the Berberidaceae; but very little attention has been given to the flowers of a surprisingly large number of the lower Dicotyledons, which in some ways are more like Monocotyledons than Dicotyledons.

An examination of the lower Choripetalae in the 'Natürlichen Pflanzenfamilien' showed that in the following families the flowers, as a rule, were built on the trimerous plan of Monocotyledons instead of the familiar pentamerous type of most of the higher Dicotyledons. The most important of these orders are the Magnoliaceae, Nymphaeaceae, Anonaceae, Myristicaceae, Lardizabalaceae, Saururaceae, Berberidaceae, Menispermaceae, Aristolochiaceae. Trimerous flowers also occur in a number of other families, although they cannot be said to be dominant. Thus in the Ranunculaceae they may occur in *Eranthis*, *Anemone*, *Coptis*, and some others, while in the Polygonaceae several genera, e.g. *Rumex*, *Rheum*, *Eriogonum*, *Koenigia*, regularly have a six-parted perianth. In short, a large number of the lower Choripetalae are characterized by trimerous flowers. Moreover, the perianth is rarely clearly differentiated into calyx and corolla, and both of these are foliar in structure, as in the Monocotyledons. This point may be of importance if the view is accepted that the corolla of the Dialypetalae is homologous with the stamens, and not composed of modified perianth segments. Whether the frequent occurrence

of trimerous carpels in so many Dicotyledons is reminiscent of some ancient trimerous ancestors is a question that might be asked.

Porsch (38) has called attention to another striking correspondence in the flowers of the Polycarpiceae and Monocotyledons which he thinks indicates a real relationship between them. He states that the nectaries in the

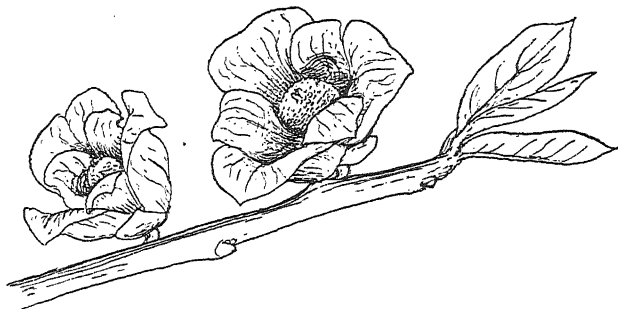


FIG. 4. Flowers of *Asimina triloba*.

two are always the product of foliar organs, most commonly the stamens, but frequently the perianth, or sometimes the carpels. This is in marked contrast with the nectaries of the Dialypetalae and Sympetalae, where as a rule they are developed from the floral axis.

So far as the floral structures of these lower Dicotyledons are concerned they more nearly resemble the Monocotyledons than they do the Dialypetalae, and this suggests the possibility that the trimerous flower is older than the pentamerous type of the majority of the more specialized Dicotyledons. Should this be true, it is conceivable that trimerous flowers were developed among the Protangiosperms, or whatever may have been the ancestors of the existing Angiosperms, and have been inherited by various phyla both among Monocotyledons and Dicotyledons.

Engler has concluded that it is not likely that such characteristically woody families as the Magnoliaceae and Lauraceae have arisen from herbaceous ancestors, or *vice versa*, but that these woody types have had quite a different origin from the herbaceous Ranunculaceae and most of the Monocotyledons, whose protangiospermous ancestors may be assumed to have been also herbaceous. Hutchinson's proposal to divide the Dicotyledons into two main series on the basis of their woody or herbaceous character, although much too far-reaching, has probably some justification.

The Leaf.

The simple, often much elongated, leaf with parallel venation, characteristic of the great majority of Monocotyledons, is by no means universal as there are many instances of broad, reticulately veined leaves exactly like those of many Dicotyledons. As examples may be cited *Trillium*, *Smilax*,

and many Araceae. The latter are of interest also in offering instances of true compound leaves, e. g. *Arisaema*, *Syngonium*, rarely met with among Monocotyledons.

A marked feature of many Monocotyledons is the great size of the

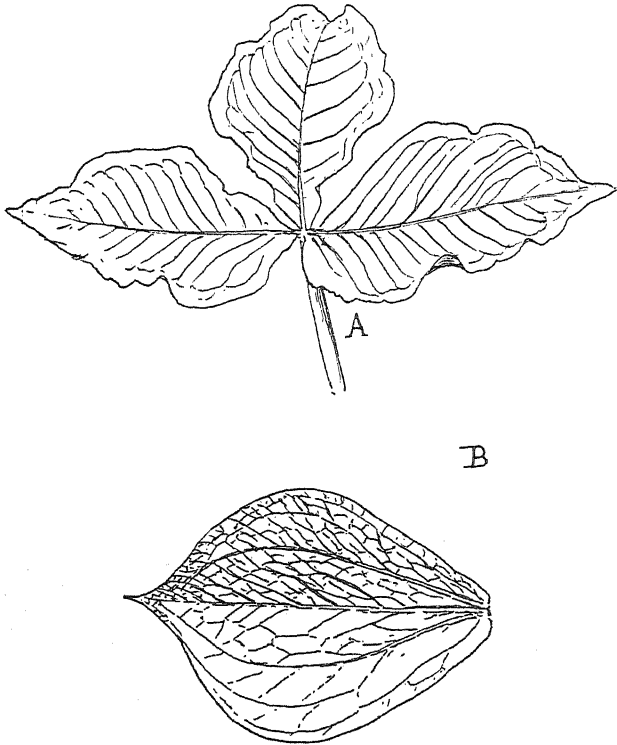


FIG. 5. A. Compound leaf of *Arisaema triphyllum*. B. Netted veined leaf of *Trillium grandiflorum*.

leaves of such forms as the Musaceae, Palms, and some of the Araceae, like *Amorphophallus titanum*, species of *Dracontium*, *Philodendron*, and *Alocasia*. The Dicotyledons can show nothing to equal some of these, and it is difficult to believe that these megaphyllous Monocotyledons can have descended from any dicotyledonous types. Their nearest analogy is with the characteristically megaphyllous Ferns, such as the Cyatheaceae and Marattiaceae. One might be tempted to suggest that this might be cited as indicating some extremely remote relationship of the Monocotyledons with the Filicineae as, like the latter, so many Monocotyledons show a marked subordination of the axis to the leaves.

It is perhaps not necessary to assume that either type of venation is the more primitive, as both are very old. Thus among the Eusporangiate Ferns both types occur. The leaflets of *Macroglossum* and *Danaea* closely resemble in their venation the leaves of the Scitamineae, while in *Kaulfussia*

(*Christensenia*) both the form of the leaf and its venation are typically dicotyledonous. Similarly, in the Ophioglossaceae, reticulate venation is found in *Ophioglossum*, while *Helminthostathiys* has parallel venation in the leaflets.

Among the Monocotyledons, *Smilax* and *Dioscorea* are among the oldest known types, and their leaves closely resemble those of such Dicotyledons as *Piper*, *Peperomia*, *Saururus*, placed by Engler at the bottom of the dicotyledonous series. *Cinnamomum*, also a very old type, and some species of *Aristolochia* have very similar leaves (see Engler and Prantl—1st Edition, III Part, first half).

The leaf of *Trillium* (Fig. 5, B), already referred to, is essentially dicotyledonous in form and venation, and this is true of many Araceae, e.g. *Arum*, *Anthurium*, *Symplocarpus*, *Lysichiton*, *Pothos*, the latter being comparable again with the *Piperales*.

Attention has been called by the writer (12) to the interesting similarity in the early leaves of certain Nymphaeaceae (Fig. 8, D) and those of *Sagittaria*.

It is very evident, then, that there is great similarity in the form and venation of the leaves between what are usually admitted to be the more primitive orders of Dicotyledons and Monocotyledons. It is much easier—and perhaps more probable—to assume that the two main leaf types, i. e. the linear parallel-veined and broad reticulately-veined, have had a quite independent origin, and have not been derived one from the other, at least not so far as concerns the Angiosperms.

The so-called 'Phyllode Theory', which has recently been taken up by Mrs. Agnes Arber (1), interprets the typical monocotyledonous leaf, not as homologous with the dicotyledonous leaf, but only with its base and petiole—comparable, therefore, to the phyllodia of certain species of *Acacia*. Difficulties are encountered, however, when one tries to apply this theory to such Monocotyledons as the Scitamineae, Araceae, and Alismaceae, where the leaf possesses a conspicuous petiole and lamina of the usual type. Equally difficult of application is it to such broad sessile, or sub-sessile, leaves as those of *Trillium* or *Smilax*. The lamina of the monocotyledonous leaf is explained as a new formation, 'pseudo-lamina', and not homologous with the leaf-lamina of the Dicotyledons. This explanation is based on the theory that the Monocotyledons are monophyletic, a view which does not at present appear probable. At any rate, the phyllode theory, even if it might be accepted for the usual monocotyledonous leaf, can hardly be applied satisfactorily to those forms in which the leaves have essentially the same structure as those of typical Dicotyledons. When we compare the leaves of *Dioscorea* and *Saururus*, for example, it is hard to believe that they are not homologous structures, and that one has a 'pseudo-lamina'; the other a true lamina.

Stem Anatomy.

The stem-anatomy of the great majority of Monocotyledons is very uniform, the cross section, with its scattered 'closed' bundles contrasting strongly with the typical dicotyledonous stem with its single circle of 'open' bundles, allowing unlimited secondary thickening.

However, as in the case of the flowers, there are many departures from the type in both Monocotyledons and Dicotyledons, and these anomalous types tend to bring the two groups together, so that it is quite impossible to find any type which is not shared by both groups.

While as a rule the vascular bundles of the Monocotyledons contain no cambium, it has been shown that traces of cambium may be present, both in the seedling and in the stem bundles of the older plant. Mrs. Arber (1) has demonstrated this in a number of genera belonging to several orders, e.g. *Hemerocallis*, *Eremurus*, *Acorus*, *Tamus*, *Phormium*. However, as is well known, where the stem shows secondary growth in thickness, as in such arborescent Liliaceae and Amaryllidaceae, as *Yucca*, *Dracaena*, *Cordylinc*, *Aloe*, *Fourcroya*, &c., this is due to the activity of a zone of meristem tissue which develops centripetally many independent closed bundles.

A considerable number of Dicotyledons show in cross section an arrangement of scattered bundles closely resembling a section of the typical monocotyledonous stem. Familiar examples of this are *Peperomia* (Fig. 6, C), *Podophyllum* (Fig. 6, B), and some other Berberidaceae, Nymphaeaceae, and certain Ranunculaceae, e.g. *Actaea*, *Thalictrum*, and some species of *Anemone* (Fig. 6, A). In these Dicotyledons, while cambium may be present, in some cases it may be entirely wanting, or so reduced as to be functionless, and such stems show no secondary growth in thickness. Holm (26), who has made a special study of *Podophyllum*, states that the vascular bundles resemble those of the Monocotyledons in the character of the phloem, and the presence of a bundle sheath, but there is evident cambium present.

Many examples of anomalous stem-anatomy occur among some of the lower families of the Dicotyledons, and to some extent are intermediate in character between the latter and the Monocotyledons. Most of these show at first the formation of a ring of typical open bundles, but the growth of these may be limited, and the further increase in thickness is due to this development of an extrafascicular zone of cambium, from which new independent bundles are developed. These may form a definite ring, as in *Abronia* (Fig. 6, D), or the broad cambium zone may form numerous scattered bundles in a manner identical with that in *Dracaena* or *Yucca*. Such a case is figured by Volkens¹ for *Eurotia ceratoides*, one of the Chenopo-

¹ Engler and Prantl. III. Theil. 1a, p. 40, Fig. 19.

diaceae, a family characterized by many deviations from the ordinary dicotyledonous type. The Nyctaginaceae, to which *Abronia* belongs, show many other analogous cases, and where there is a permanent woody stem

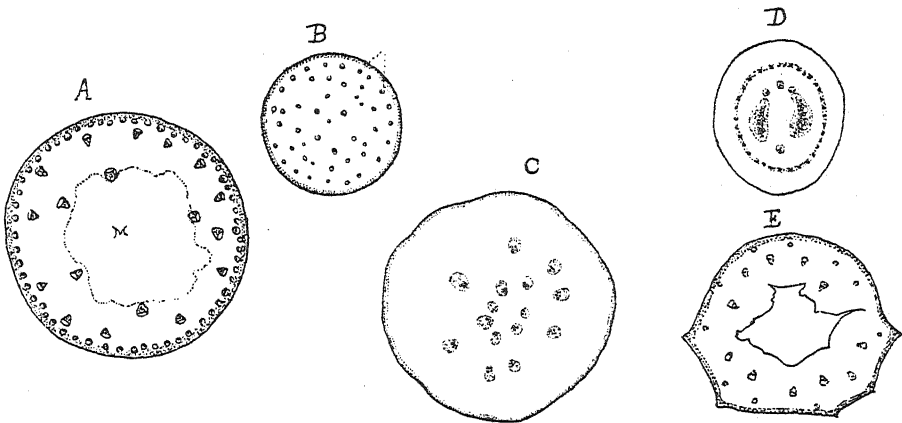


FIG. 6. Cross-sections of stems. A. *Anemone Japonica*. B. *Podophyllum peltatum*. C. *Peperomia trinervis*. D. *Abronia umbellata*. E. *Narcissus poeticus*.

this is the result of the activity of the extrafascicular cambium zone in which are developed successive rings of bundles separated by ground tissue. These bundles are incapable of any secondary growth.

For further details of these anomalous stem structures the reader may be referred to De Bary's classical volume.¹

Should we attempt to derive one type of stem structure from the other we might assume that these anomalous structures lead up from the monocotyledonous type with its scattered closed bundles to the Dicotyledons with their single circle of open bundles; or the latter might be considered as primitive, and the monocotyledonous type reduced from it. One explanation is perhaps as good as the other.

Whether we accept either of these, or neither, it is pretty evident that it is quite impossible to separate absolutely Dicotyledons and Monocotyledons on the basis of their stem-anatomy.

In connexion with the question of the possibility of a direct origin of the Angiosperms (or Protangiosperms) from the Eusporangiatae, it might be noted that within the family Ophioglossaceae the structure of the stem in *Ophioglossum* might be compared with that of a Monocotyledon, while in *Botrychium* the stem structure is almost exactly that of a woody dicotyledonous axis.

¹ De Barry, A.: Vergleichende Anatomie der Vegetationsorgane der Phanerogamen und Farne. Leipzig, 1877.

The Embryo.

Although the development of the embryo is more or less completely known in a good many Monocotyledons, there is still much to be learned about the development in a number of the lower families, especially the Palms.

The degree of development in the ripe seed differs greatly. In much the larger number the embryo remains relatively small, and is surrounded by abundant endosperm. In extreme cases, as in the Orchidaceae, the embryo may be reduced to a quite undifferentiated body composed of only a few cells. Between this and the large completely differentiated embryo of such forms as *Alisma* and *Naias*, where the embryo completely fills the seed, all stages of development may be found.

In most of the current texts the embryo of *Alisma*, based upon Hanstein's (21) investigations, serves as a type for the Monocotyledons, although the Alismaceae differ in several respects from most of the more specialized families, e. g. Liliaceae, Iridaceae, Orchidaceae, in which the embryo is much less advanced in the ripe seed.

The cotyledon in *Alisma* appears to be a truly terminal structure, and the same is true in *Sagittaria* (42) and *Naias* (7). The origin of the organs of the embryo in the latter was studied by the writer. In *Naias* the cotyledon arises from the terminal segment of the young embryo, while the stem apex is developed from the second segment and is lateral in position. This type of embryo seems to be widespread among Monocotyledons, and is generally accepted as typical of the whole group. However, there are a good many exceptions.

Solms-Laubach (45) found in some of the Dioscoreaceae and Comelynaceae that the stem apex was terminal and the cotyledon lateral, a condition, in a way, intermediate between the 'typical' monocotyledonous embryo and that of the Dicotyledons. A similar condition is found in the young embryos of *Zannichellia* (Fig. 7, A), and probably will be found in other Monocotyledons.

As in the dicotyledonous embryo there is in most Monocotyledons a conspicuous primary root, which is later replaced by numerous secondary ones.

Coulter (18) found that in *Agapanthus* the embryo sometimes developed two cotyledons. The young embryo showed a peripheral cotyledonary zone, which might give rise to either one or two growing points, which would develop into either one or two cotyledons. There is no stem apex at this time, and the early leaves are developed independently, suggesting the condition in *Ophioglossum Moluccanum*.

Where endosperm is present the cotyledon serves as a haustorium, and sometimes, as in the Grasses and Palms, develops a special organ, the

scutellum, which remains permanently within the seed, and only the basal portion of the cotyledon emerges as a sheath within which the plumule is

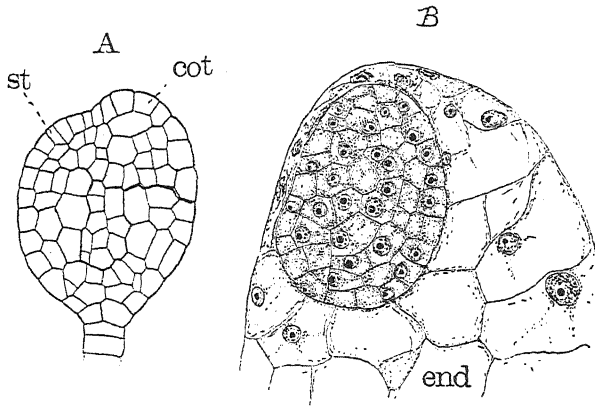


FIG. 7. A. Embryo of *Zanichellia palustris*: st., stem, apex; cot., cotyledon. B. Embryo of *Lysichiton Kamchatcense*, surrounded by endosperm, end.

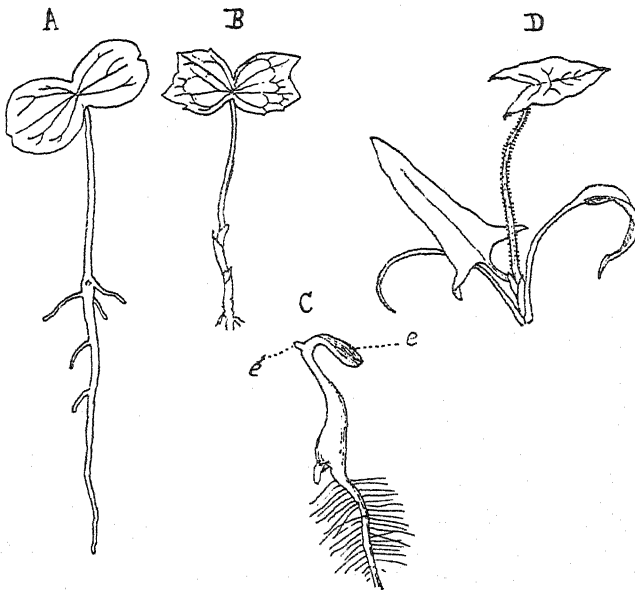


FIG. 8. A. Seedling of *Podophyllum peltatum*, showing the united cotyledons. B. Seedling of *Podophyllum*, the second year, showing several scale-leaves and the bifid foliage leaf. C. Seedling of *Abronia umbellata*, showing the very unequal cotyledons, e, e. D. Seedling of *Victoria regia*, showing the sagittal early leaves. A, B. After Holm. C. After Klebs. D. Goebel, after Trécul.

borne. Where no endosperm is present, the cotyledon develops at once as the first foliage leaf. In a very large number of other cases the cotyledon is finally withdrawn after the endosperm is exhausted, and becomes the first green leaf. This type of embryo is probably more primitive than that

in which the cotyledon is specially modified as a haustorium, as in the Palms and Grasses.

It is impossible to decide which is the more primitive condition—the highly-developed embryo completely filling the ripe seed, or the small embryo with abundant endosperm. The prevalence of the former type in many of the simpler Monocotyledons, e. g. *Naias*, Alismaceae, *Potamogeton*, &c., might be so interpreted, but on the other hand the lowest order, in the Engler system, Pandanales, is characterized by abundant endosperm. The Araceae show great variation in this respect, some having endosperm, others not.

While the great majority of Dicotyledons show the familiar two cotyledons on opposite sides of the terminal shoot apex, there are a number of 'pseudo-monocotyledons' among them, which suggests, at any rate, the possibility of a condition intermediate between the Dicotyledons and true Monocotyledons, comparable to that of the terminal stem apex and lateral cotyledon in the Dioscoreaceae and Commelynaceae. Among the Dicotyledons with a single cotyledon may be mentioned *Cyclamen*, *Abronia* (Fig. 8, c), *Ranunculus Ficaria*, *Corydalis* spp. In these there is either a more or less complete suppression of one cotyledon or a retardation in the growth of one of them, as if they were not of equal age. This is very marked in *Abronia*, a genus belonging to the Nyctaginaceae, a family characterized by other anomalous structures, e. g. the stem anatomy and the monochlamydeous coloured perianth, both somewhat reminiscent of the Monocotyledons.

Among the *Ranales* are several genera in which the embryo shows certain peculiarities suggestive of the Monocotyledons. Thus it has even been claimed that the Nymphaeaceae are Monocotyledons, and should be removed from the Dicotyledons. Lyon (34), from a study of the embryo of *Nehumbo*, reached this conclusion, and Cook (16) states that in *Nymphaea odorata* and *Nuphar advena* the embryo is really monocotyledonous.

Hill (23) has found in several geophilous species of *Peperomia* that one cotyledon remains within the seed as a haustorium, the second appearing above ground as the first green leaf.

A number of Ranunculaceae, e. g. *Actaea*, *Delphinium nudicaule* (Fig. 9), *Eranthis*, have the cotyledons united into a tube, much as in many Monocotyledons, with the plumule at the bottom of the tube. In *Delphinium nudicaule* even the bases of the two laminae are united. A similar condition exists in some Berberidaceae. In *Podophyllum* (Fig. 8, A) the seedling is much like that of *Delphinium nudicaule*, but no leaves except the cotyledons are developed the first season. The second season the terminal bud, after producing several scale leaves, gives rise to a single bi-lobed foliage leaf, which resembles to an extraordinary degree the united pair of cotyledons. Examination has been made of the embryos of several

of these anomalous Berberidaceae and Ranunculaceae (32, 35), and it has been shown that the formation of the cotyledons begins as a crescent-shaped primordium, very much as in the typical Monocotyledons and



FIG. 9. A. Seedling of *Delphinium nudicaule*, showing united cotyledons.
B. An older stage, the first leaf breaking through the cotyledonary tube.

Nelumbo. The margin of this ridge becomes notched, to form the two cotyledon-laminae, the basal portion giving rise to the tube. Whatever may be the explanation of this, it is clear that in these cases the two cotyledons arise from a common primordium, and are not formed independently on opposite sides of the stem apex.

Miss Sargent (1) has objected that the admission that the cotyledon of the Monocotyledons is really terminal would imply that it was not homologous with a true leaf, which, apparently, she believes must always be secondary to the axis. However, the terminal origin of the primary leaf in *Ophioglossum moluccanum* is unmistakable and entirely independent of the stem apex, which is not developed until several independent leaves have been formed. The early leaves of other Eusporangiateae are also quite independent of the stem apex, and the same is true of *Isoetes*. As these have been thought to be remotely related to the Monocotyledons, the terminal position of the cotyledon might be used as an argument in favour of this theory.

Whether we accept the view so ably defended by Miss Sargent, that the monocotyledonous embryo is derived from a Dicotyledon by the fusion of the two cotyledons of the latter, or whether the two cotyledons result

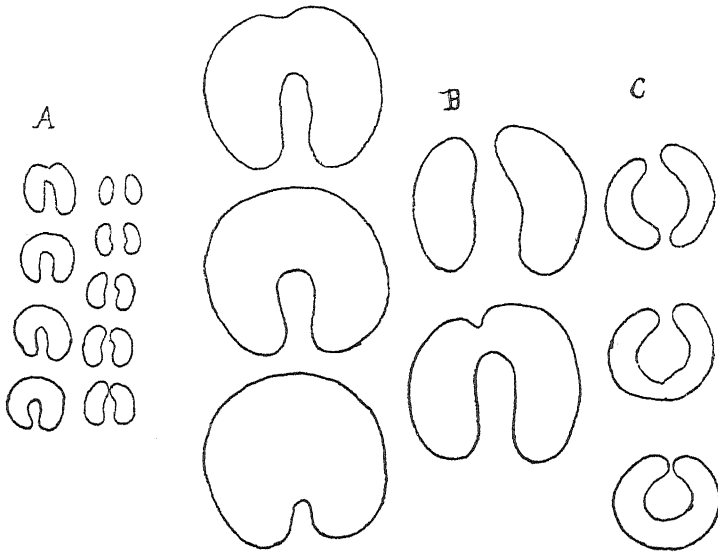


FIG. 10. A. Series of transverse sections through the cotyledonary region of the embryo of *Podophyllum*. B. Similar series from *Actaea alba*. C. Similar series from *Delphinium tricornis*. A. After Lewis. B, C. After Mottier.

from a bifurcation of the cotyledon of a monocotyledonous ancestor, or whether, as seems to the writer more likely, the two types of embryo have arisen independently in several phyla, the differences between them are not fundamental.

The Female Gametophyte.

While most of the Angiosperms show great uniformity in the structure of the embryo sac there are numerous departures from this, especially among the lower Monocotyledons. While Engler assigns to his Protangiosperms the 8-nucleate type considered typical for Angiosperms, it is noteworthy that the two orders, *Pandanales* and *Piperales*, which he cites as most nearly approximating his Protangiosperms, contain genera, viz. *Pandanus* and *Peperomia*, whose embryo sacs differ greatly from the 8-nucleate type. In both of these there is a marked increase in the number of nuclei, which in *Pandanus* (13) may exceed sixty at the time of fertilization (Fig. 11, A, B). It seems much more likely that the ancestors of the Angiosperms had a much more developed female gametophyte than the 8-nucleate embryo sac of most existing forms; and it seems quite justified to interpret such a gametophyte as that of *Pandanus* as an approach to the condition in the Protangiosperms, or whatever may have been the ancestors of these existing Angiosperms.

A greater or less increase in the tissue of the female gametophyte has been found in a surprisingly large number of the lower Monocotyledons. This is mainly in the antipodal region where the increase in the number

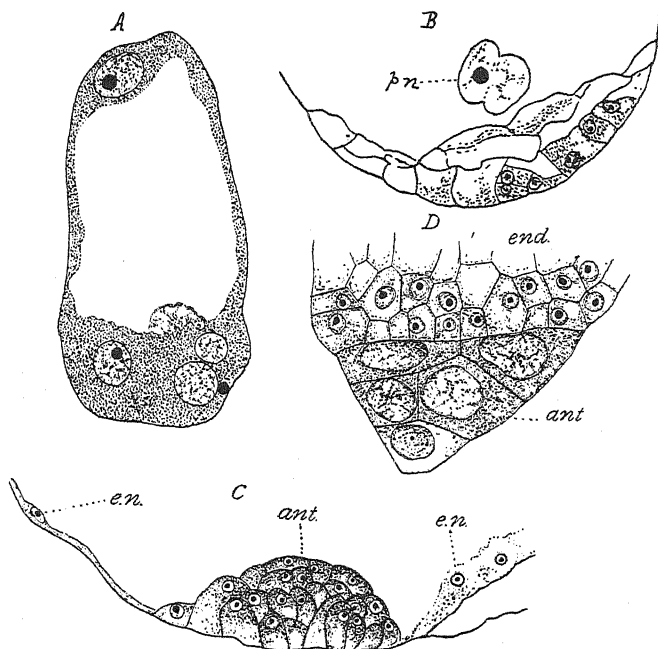


FIG. 11. A. Young embryo-sac of *Pandanus Artocarpus*. B. Antipodal region of an older sac; *pn.*, two of the polar nuclei fusing. C. Antipodal cells of *Sparganium simplex*. D. Antipodal cells of *Lysichiton Kamtschaticense*.

of the antipodal cells may be very marked. Cannon (15) has found thirty-six or more antipodal nuclei in *Avena fatua* at the time of fertilization, and in general the Gramineae show marked increase in the number of antipodals. In other cases, where at the time of fertilization the usual three antipodals occur, there may be a greater or less increase subsequent to fertilization; and the increased antipodal tissue is undoubtedly important in the further development of the gametophyte. The most striking case is that of *Sparganium* (9), where there may be as many as 150 antipodals (Fig. 11, C). Often there is great increase in the size of the antipodals with fewer divisions, but evidently they are functionally important. In the latter category may be placed *Naias*, *Zannichellia*, *Lilaea*, *Triglochin* (24), *Lysichiton*, and other Araceae (Fig. 11, D). It is highly probable that further investigation will greatly increase this list.

This marked tendency among so many of the simpler Monocotyledons to increased development of the vegetative tissues of the gametophyte might be used as an argument in favour of their primitive rather than derivative character.

Are the Monocotyledons, as a whole, a primitive or derivative group? We have seen that the majority of students of this question are in favour of the view that they have been derived from Dicotyledons, and that their predominantly herbaceous structure is the result of their adoption of an aquatic or geophilous habit—the latter being the result of cold or dry conditions.

However, when we realize that aside from the Grasses, Sedges, Liliiflorae, and some aquatic families, the Monocotyledons are especially characteristic of the humid tropics, this explanation is not entirely satisfactory. The geophilous habit of the Musaceae, Araceae, Zingiberaceae, and many Orchidaceae, so characteristic of the humid equatorial forests, hardly harmonizes with this theory. Is it not quite as likely that this is a primitive character inherited from some herbaceous ancestors, Protangiosperms or Filicineae? The latter are predominantly herbaceous and geophilous, while the existing heterosporous genera, some of which, especially Isoetes, show interesting suggestions of affinity with the Monocotyledons, are all either aquatic or amphibious.

The marked preponderance of Monocotyledons in the tropics suggests that they may have originated under similar conditions. In some recent papers (5), Bews brings forward evidence to show that the Angiosperms probably originated at a time when the climate was similar to that in the existing lowland tropics. He claims that the present tropical forests of South Africa, a very old region geologically, where presumably the climate has changed but little during a very long period, show a larger percentage of primitive types than are found in the forests of cooler regions; and believes that the temperate and boreal types have been developed secondarily from tropical ancestors as the result of climatic changes largely due to extensive mountain formation and adaptation to the lower temperature of the higher elevations.

If his view is correct, the many Monocotyledons characteristic of tropical lowlands, especially the forest margins and shores of rivers and lakes, might well be ancient forms and not secondary types, as now believed by many botanists.

As to the relative antiquity of Dicotyledons and Monocotyledons, the geological record is too incomplete to give much information. Both occur in the Lower Cretaceous in forms much resembling existing genera, indicating that true Angiosperms must have existed at a very much earlier period. Should the recent discovery of alleged monocotyledonous fossils from the Carboniferous (25, 37) prove correct, this would of course make these very much older than any known Dicotyledons.¹

It is generally believed that during the Jurassic and early Cretaceous, when it is supposed Angiosperms first became prominent, a much more uniform climate prevailed than at present, and presumably warm if not

¹ It has since been shown that these fossils are the well-known *Myeloxylon*.

tropical conditions. This would be quite in harmony with Bews' conclusion that the Angiosperms originated under tropical conditions. The predominance of Monocotyledons in tropical regions to-day might then indicate that they are relict forms rather than secondary ones, and their simpler structure is not the result of reduction from dicotyledonous ancestors.

The similarity to Monocotyledons shown in certain Dicotyledons, already referred to, has not perhaps received adequate attention. It is significant that in these monochlamydeous and Ranalean flowers the floral envelopes are with few exceptions obviously of foliar nature, as they are in the petaloidous Monocotyledons. This would indicate a possible derivation from a common stock with three-parted floral envelopes, i.e., the trimerous floral type is older than the pentamerous one so prevalent among Dicotyledons. It is also interesting to note that the anomalous stem structures among the Dicotyledons are for the most part associated with flowers approximating the monocotyledonous type.

The relative numbers of the two groups and their distribution might also be cited as indicating that the Monocotyledons represent a relict group rather than a comparatively recent one derived from Dicotyledons.

The latter greatly outnumber the Monocotyledons, having forty-one orders with over 100,000 species, while the Monocotyledons, with only eleven orders, have only about one-third as many species (20). There are among the latter many highly specialized, and probably recent forms, like the Orchids and some Iridaceae and Scitamineae, and these specialized forms comprise a very large majority of the existing species. The Orchidaceae, for example, have some 17,000 described species, very nearly half of all known Monocotyledons. If to these we add the large orders Liliiflorae, Farinosae, and Scitamineae, which comprise most of the showy-flowered and presumably more modern and specialized floral types, the number of species remaining is less than one-third of the total, and more than half of these belong to the Glumiflorae which, although evidently old types, have adapted themselves to modern conditions, and compete on even terms with the dominant Dicotyledons, the only Monocotyledons except some aquatics which have been able to do so.

This paucity of species and lack of adaptability certainly are suggestive of an ancient rather than a modern type.

Whether we are to assume that the Angiosperms are descended from Protangiosperms, Gymnosperms, or directly from Pteridophytes, must remain for the present unanswered.

In view of our present knowledge, we are probably warranted in assuming that the Monocotyledons are as old—possibly older—than the Dicotyledons, but do not form a single closed phylum. Coulter (17), in his very thorough analysis of the inter-relationships of the Monocotyledons, expresses the view that the three orders, *Pandanales*, *Helobiales*, and

Glumales, represent three independent stocks from which the other orders may have been derived. Thus he associates with the *Pandanales* the Palms and *Synanthales*; with the *Helobiales* the *Arales*; and with the *Glumales* (Glumiflorae) the remainder of the Monocotyledons. Such a conclusion is of course merely tentative, but probably comes as near to the truth as we can expect at present.

The structural differences between Monocotyledons and Dicotyledons as a whole do not seem sufficient for the establishment of two co-ordinate sub-classes. It will probably be necessary to recognize a much larger number of independent phyla (sub-classes?), some Monocotyledons, others Dicotyledons. Perhaps further studies on some of the anomalous Dicotyledons may show that they are really more nearly related to certain Monocotyledons than to the other Dicotyledons, and should be united with the former as a separate group.

The possibility of some of the structural resemblances between Monocotyledons and Dicotyledons being homoplastic rather than really homologous is a question which might well be investigated, and further studies in this direction may throw some light upon the vexed question of the interrelationships of the two. For instance, it would be interesting to know how far the anatomy, floral structures, embryo-sac and embryo are correlated in the lower orders of both groups.

At present one can do little more than offer suggestions; but it is hoped further efforts will be made toward establishing a more scientific classification than that now in general use.

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Cladites bracteatus, a Petrified Shoot from the Lower Coal-measures.

BY

D. H. SCOTT, F.R.S.

With Plates XVII and XVIII and five Figures in the Text.

THE fossil to be described is a specimen from Shore, Littleborough, the sections of which were received from Mr. James Lomax as long ago as 1904. It is a small but stout shoot, with crowded leaves; so much is obvious at a glance (Pl. XVII, Fig. 1 and Text-fig. 1). The affinities of the fossil have puzzled me from the first, and are still undetermined. Owing to this uncertainty I have long delayed describing it. Now, however, it seems desirable that the facts should be published, for the specimen, so far as I know, is unique.

The fossil is from a roof-nodule, as shown by the *Goniatite* shells associated with it. The preservation, as is usual in specimens from such a source, is patchy, and is nowhere specially good. Much of the structure, however, can be made out sufficiently well.

The total length of the most complete section is 4.7 cm.¹ The shoot is flattened; its smaller diameter (including the leaves) is about 2.75 cm. The greater diameter could not be measured, as no section in that plane is complete. The flattened *axis* of the shoot measures about 3.5 cm. by 1 cm.

Fifteen sections were supplied by Mr. Lomax; so far as I know, no others exist.

The sections were cut as follows:—

Slides 1–7, longitudinal sections parallel to the *shorter* diameter of the flattened shoot. Of these no. 3 is the most median.

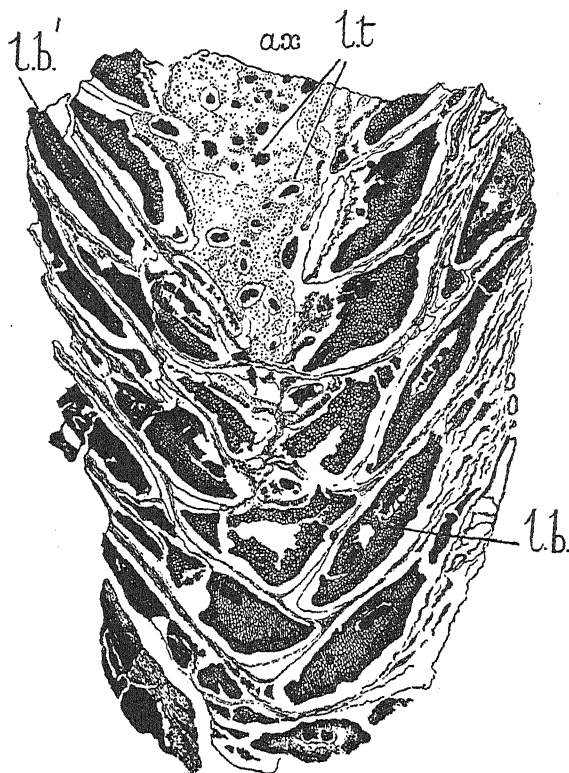
Slides 8–11, transverse sections, showing more or less of the axial wood. No. 11 is the most extensive.

Slides 12–15, longitudinal sections parallel to the *longer* diameter of the flattened shoot. No. 15 is the most median, showing much of the axial wood.

¹ The actual length of the original specimen was perhaps twice as great, for the three series of sections must have been cut from different parts of the fragment.

GENERAL DESCRIPTION.

The stout axis is closely set with small, simple leaves, which appear to be spirally arranged (Pl. XVII, Figs. 1 and 2; Text-fig. 1). There is no indication of any other appendages, reproductive or otherwise. The



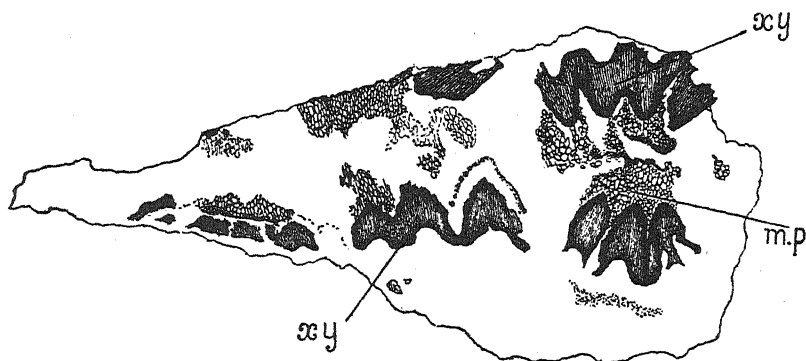
TEXT-FIG. 1. Tangential section of the shoot. *ax.*, axis, only shown in upper part of section; *lt.*, leaf-trace strands in cortex; *l.b.*, *l.b.'*, bracts. Section cut parallel to smaller diameter (Slide 4). $\times 2\frac{1}{4}$. G.T.G.

specimen, therefore, cannot itself be a cone, though it might be the stalk on which one or more cones were borne. The leaves do not show the structure of foliage-leaves, but rather suggest bracts or scale-leaves (Text-fig. 5). The shoot can, therefore, hardly be an ordinary vegetative branch. As there is no sign of young organs or tissues, it cannot be a bud. We may conjecture that it may probably represent the lower part of a fertile shoot, which may have borne reproductive organs at a higher level.

The axis contains a large stele, much flattened like the whole shoot. The stele is never shown complete, but in the best transverse section (No. 11) the part remaining measures 2.6 cm. (incomplete) by 0.7 cm., to the outside of the wood, which is alone preserved. In this case sixteen

woody wedges (or portions of them) are shown in two rows, no doubt the remains of a flattened ring enclosing the pith (see Text-fig. 2). The wood is dense and almost entirely secondary, with apparent rings of growth.

The leaves, which may provisionally be termed *bracts*, are about 1.7 cm. in extreme length. The general form was probably lanceolate, but this is



TEXT-FIG. 2. Transverse section of the flattened axis. *xy.*, wood, much broken up; *m.p.*, mixed pith, or central tissue (Slide 11). $\times 3\frac{1}{2}$ G.T.G.

not well shown in the sections. They are diamond-shaped in transverse section at the base (Pl. XVII, Fig. 2) becoming more lenticular further out (Text-fig. 5).

The tissue of the bracts is brown in colour and uniform; there is no marked differentiation in the mesophyll. Each bract contains a very large median vascular bundle near its upper surface. This is accompanied by much smaller lateral strands. All the foliar strands have a stele-like structure, with much secondary xylem. The large median bundle appears to have been formed by the fusion of several leaf-trace strands, while the lateral bundles branched off from the main one.

THE AXIS.

The structure of the axis of the shoot is best shown in the transverse sections. Most of the longitudinal sections also show something of it, but usually cut tangentially; only two longitudinal sections (Nos. 3 and 15) show anything of the wood.

The axis is completely clothed by the contiguous bases of the bracts, so that no free surface is left (Pl. XVII, Fig. 2). The cellular structure of the primary cortex is not actually preserved, though a number of small, dark masses no doubt represent the altered cell-contents. In the region of the inner cortex even these may be absent. The leaf-traces, however, traversing the cortex, are preserved, at least in the outer zone.

At the external border of the axis, adjacent to the bract-bases, there

are distinct remains of a periderm, several cells thick. The cells have the usual radial seriation and tangential walls (Pl. XVIII, Fig. 12).

The stele, as already mentioned, is nowhere shown complete. The best transverse section (see Text-fig. 2) contains part of the flattened ring of wood, with the outer portion of the pith or central tissue. The wood consists of a series of bundles or wedges, all no doubt originally continuous with one another, though now sometimes accidentally torn apart. Thus the outline of the woody zone is crenulated, the bays on the inner side being very marked. On the outer edge the prominences correspond to the bays of the inner margin, and *vice versa* (Pl. XVII, Fig. 3).

The radial depth of each woody wedge in the median line is about 2 mm. The extreme depth, measuring from prominence to prominence, is 2.5 mm. or more. The tangential width of each wedge is about 2 mm.

The whole of the woody zone has its elements in radial seriation and appears to be secondary (Text-fig. 3). I have sought in vain for any distinct primary xylem. Some elements, somewhat larger than the secondary tracheides, are present in the outer angles of the bays, but they have proved to belong to the central tissue and not to be of the nature of primary xylem. The structure of the wood, in fact, is in this respect that of a typical coniferous stem, in which there is no obvious distinction, in transverse section, between primary and secondary xylem.

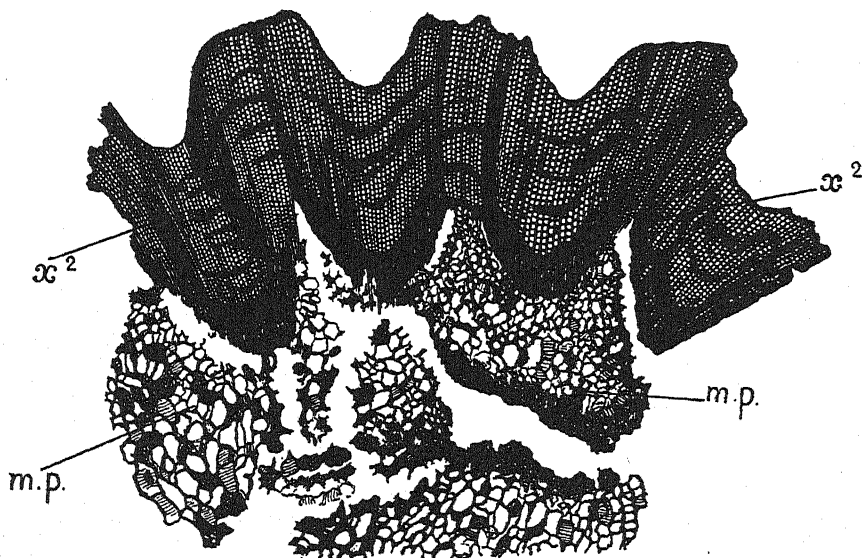
The wood is very dense, with small tracheides and narrow rays. The tracheides are commonly about $24\ \mu$ to $30\ \mu$ in diameter. In all the sections apparent rings of growth are evident (Pl. XVII, Fig. 3). There are several of them; they are fairly continuous, with an undulating course, corresponding to the crenulated outline of the wood. The bands indicating the zones are little differentiated; the appearance is sometimes due to a single layer of narrow tracheides, sometimes to a more gradual change in their radial diameter. It is not at all likely that these zones of growth have anything to do with annual rings.

No phloem is preserved. The remnants of tissue sometimes found just outside the wood only differ from the disintegrated cortex in the somewhat smaller size of the dark bodies (cell-contents?) (Pl. XVII, Fig. 3).

The longitudinal sections of the wood leave much to be desired. The most extensive is that in Slide 15, where a considerable tract of wood is seen in tangential section (Pl. XVII, Fig. 4). It appears from this that the woody wedges form a network. As the section is somewhat oblique to the axis, it touches on the central tissue at one end of the wood (the lower) and on the disorganized cortex at the other. In the thinner parts the medullary rays are well shown (Pl. XVII, Fig. 5); they are uniseriate, commonly only one cell in height, but sometimes more, seven cells being the greatest number observed. The tracheides show no distinct pitting on their tangential walls; in fact in the outer layers tangential pits were generally absent, an important point

for comparison with other stems. As the plane of section approaches the inner margin, spiral markings begin to appear, as would naturally be expected.

There is no good radial section of the wood. In Slide 3, where a small portion of wood is shown, the plane approaches the radial at one place



TEXT-FIG. 3. Part of stele, enlarged. x^2 , the wood, apparently all secondary; *m.p.*, mixed pith or central tissue. On many of the cells the barred sculpturing is indicated (Slide 11). $\times 15$. G.T.G.

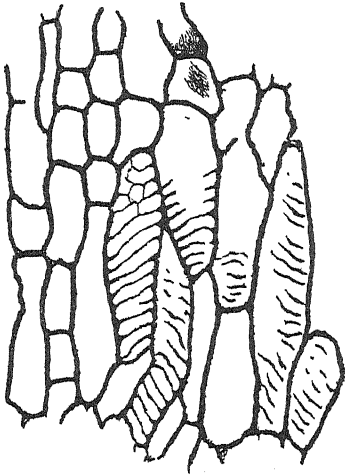
(Pl. XVII, Fig. 6) and the pitting can be observed, though obscurely. It appears to be scalariform; at any rate the pits are much elongated transversely. No multiseriate pitting has been detected.

At the inner margin of a woody wedge, where it projects furthest into the central tissue, elements with very lax coils, spiral and annular, have been observed, and presumably constitute the protoxylem. Spiral tracheides, however, occur at all points of the inner margin. There can, in any case, be no doubt that the xylem was *endarch* in its development.

The indications of leaf-traces found in the wood will be considered later, when the course of the leaf-traces is discussed.

A certain amount of the central tissue, internal to the woody zone, is preserved, but only the peripheral part. Assuming that the wood formed in life a more or less circular ring, a great amount of tissue in the middle of the stem must have entirely perished. It is, of course, possible that the stele was not circular; an analogy for a naturally flattened shoot and stele is to be found in the fertile branches of Cordaites, such as *Mesoxylon multirame* (5). But even if that were the case, it is evident that a considerable part of the central tissue has been lost (Text-fig. 2).

The remaining portion has a peculiar structure. Its cells have the appearance of short tracheides, the walls being conspicuously barred, with the bars rather far apart (Text-figs. 3 and 4). Sometimes the sculpturing takes a spiral form. The elements are often somewhat elongated in the



TEXT-FIG. 4. A few cells of the central tissue. Where the walls are seen in surface-view the sculpturing is shown (Slide 11). \times about 100. G.T.G.

radial direction, but frequently undergo division into short cell-rows. The dimensions of the larger cells are often about 280 by 120μ as seen in transverse section. In longitudinal section they are short, measuring about 120 – 200μ . In the bays of the wood the elements of the central tissue are smaller, but appear to be of the same general shape. It is doubtful whether any purely parenchymatous unsculptured cells exist in the central tissue as preserved.

How are we to interpret this tissue of barred elements? It might no doubt be compared with the central region of the wood in *Lepidodendron selaginoides*, where short tracheides occur. In that plant, however, the central tracheides, though short, are often somewhat elongated vertically, and transitional forms connect them with the surrounding zone of normal primary xylem. In our *Cladites* there is no such zone present; the barred elements are not vertically elongated and show no transition to xylem-structure. I should be more inclined to compare this tissue with the central mass of short tracheide-like elements, which replaces the pith in the genus *Bilignea* (9). In the *Cladites* it is, of course, possible that the zone of barred elements may have surrounded a true, parenchymatous pith which has wholly perished.

THE BRACTS.

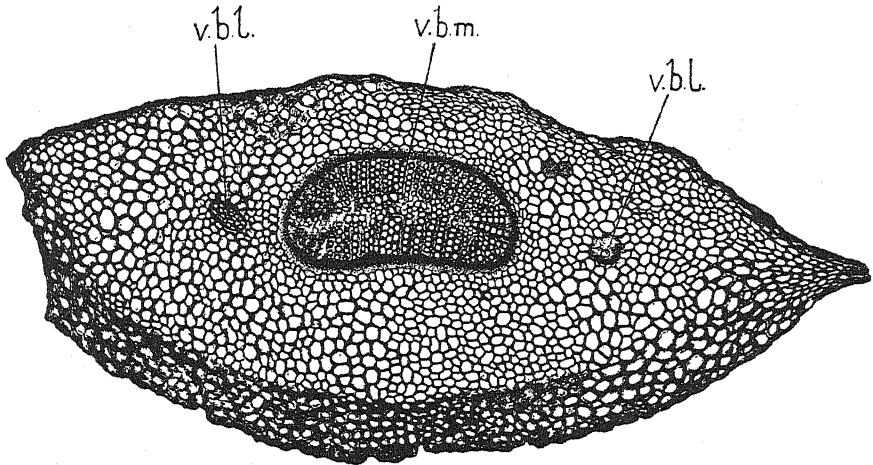
A tangential section, through the bases of the bracts, is shown in Pl. XVII, Fig. 2. The bracts, as already mentioned, are diamond-shaped at this level; they fit closely together, leaving no free surface of stem.

The typical structure of a bract, when free from the stem, is represented in Text-fig. 5. The section was cut fairly near the base, but at a point where the sectional form has become lenticular (see also Pl. XVIII, Figs. 7 and 8).

The tangential, diamond-shaped section of a bract-base may measure as much as 1.4 cm. horizontally, by about 1 cm. vertically. If cut a trifle further out, a slight wing appears at each side. The tissue has usually contracted so that it no longer fills the contour of the bract. It is probable

that in this contraction the epidermis was left behind. It is nowhere clearly shown.

The ground-tissue of the bract consists of very uniform parenchyma, with short cells, so that there is not much difference between its aspect in transverse and longitudinal section. The peripheral layers, especially on



TEXT-FIG. 5. Transverse section of a bract showing the fairly uniform ground tissue. *v.b.m.*, the large, stele-like, median bundle; *v.bl.*, the two lateral bundles (Slide 7). \times about 20. G.T.G.

the lower side, are thicker walled than the rest (Text-fig. 5; Pl. XVIII, Figs. 7 and 8), but otherwise there is no differentiation. In rare cases the cells towards the lower surface show an arrangement in vertical rows, as if a superficial periderm had formed. More frequently an internal periderm, cutting deeply into the tissue, even as far as the vascular bundles, has been observed. But such formations in the bracts are inconstant, and may well have been traumatic in origin.

The vascular system of the bract, in its typical form at a short distance above the base, is shown in Text-fig. 5. It consists of a very large median bundle with a small strand on either side. The vascular strands always lie near the upper surface. The large median strand is transversely elongated. In the case figured it measures 2 mm. in length by about 0.75 mm. in breadth, and this is a usual size, in this region. Its structure is that of a stele, not of an ordinary foliar bundle. The wood is for the most part secondary, the regular rows of tracheides radiating in all directions. In the middle is a narrow band of irregular elements, presumably representing the primary xylem. The phloem, which no doubt surrounded the wood, is never clearly preserved.

The longitudinal sections of the foliar bundles are always obscure. Scleriform markings are shown, and probably characterized the mass of

the xylem. Annular and spiral elements also occur, which one supposes belonged to the primary portion, but it is impossible to localize them. Apparently the structure of the wood of the main bract-bundle was similar to that of the stelar xylem of the axis.

All the foliar bundles, even the smaller, and also all the leaf-traces passing through the cortex, show the same apparently stelar and concentric structure as the main strand. The smaller bundles, however, are usually approximately circular in transverse section.

The lateral strands are given off from the ends of the elliptical main bundle, not always at the same level (see Pl. XVIII, Fig. 7). There is evidence that the lateral bundles branched, at least once, so that in the more distal parts of the bract there must have been at least five strands, the large median one, and two smaller on either side.

It will be noticed that the vascular system of these organs is quite unlike that of any known leaf of Palaeozoic age.

THE LEAF-TRACES.

We have now to ascertain how this peculiar structure is attained, or in other words, to follow, if we can, the course of the leaf-traces. We have already seen that the lateral strands of the bract branch off from the large median bundle. Hence it is only with the source of the latter that we are concerned. The problem does not admit of a complete solution from the material at hand, for the strands supplying the bracts cannot be followed continuously all the way from the stele. Little or nothing is to be seen of them in the region of the inner cortex.

It is a fortunate circumstance that the tangential sections of the shoot are not cut perfectly parallel to the axis, but somewhat inclined to it, so that as we follow the section from end to end we gradually pass from a shallower to a deeper plane or *vice versa*. This greatly assists in determining the behaviour of the leaf-trace strands.

For example, we may take the most median section of the series cut parallel to the smaller diameter of the flattened shoot (Pl. XVII, Fig. 1). The section cuts deepest at the top where, as already mentioned, the wood is reached. Following the axis downwards we find, in the middle region, a blank space, representing the inner cortex, where no tissue is preserved. Lower down, the sloping plane of section reaches the outer cortex, and here we meet with numerous round strands, the leaf-trace bundles, cut transversely on their outward course. They are at first somewhat irregularly grouped, but about three-fifths of the length from the top, we come to a definite inclined row of four strands, lying in a comparatively clear space (Pl. XVIII, Fig. 9). This, we may conclude, constitutes the vascular supply for one bract. So far there is no marked increase in the size of the strands.

Still lower down (and therefore further out) we come to definite *pairs* of bundles; note especially a pair in the median line (Pl. XVIII, Fig. 9). Here the strands are much larger than before—evidently fusion has taken place, combining the four strands into two. Finally, at the bottom, where the actual base of a bract is reached, it contains the one very large bundle, with which we are already familiar, the ultimate product of fusion (Pl. XVII, Fig. 1; Pl. XVIII, Figs. 7, 8).

This conclusion is confirmed by the other sections, but there is some variation in the details of the fusion process. In some cases three bundles are seen about to enter a bract. This may be due to irregularities in the fusion. Other good examples of the two strands entering the base of a bract are shown in Pl. XVIII, Figs. 7 and 8, together (in Fig. 7) with a 3-bundle stage, just referred to. The case, shown in Pl. XVIII, Fig. 7, indicates that after the leaf-trace strands have united to form two only they continue to increase in bulk, as followed outwards, for the outer of the two pairs shown has much thicker strands than the inner.

So far then, it appears that the supply to a single bract consists of about four strands, which, as they enter the base of the organ, fuse into two (a stage of three sometimes intervening), and these two finally unite to form the single large median bundle of the bract. This in turn gives off lateral branches to supply the marginal parts of the appendage.

The question now arises, how are the leaf-trace strands derived from the stele? This part of their course is impossible to observe, for, as we have already seen, the region of the inner cortex is not preserved, so that there is a serious gap in following the course of the leaf-traces. In the wood itself, however, the starting-points of these strands can be detected. In a tangential section, leaf-traces, passing through the wood, can easily be seen (Pl. XVII, Fig. 4 and Pl. XVIII, Fig. 10). The leaf-trace is indicated by a small gap in the wood. The actual strand lies at the lower edge of the gap in continuity with the stelar wood. The gap is usually traversed by a few irregular shreds of disorganized tissue.

The leaf-trace strands are not paired or in any way grouped; there is no indication of a double or multiple trace, and the inference is that the trace, in starting from the wood, was a single bundle only. In one case, in a transverse section, some tracheides are seen in longitudinal aspect running in a radial direction (Pl. XVIII, Fig. 11). It is probable that they represent a leaf-trace. The gap in the wood on which the tracheides border is of course accidental. The tissue thus being in a damaged condition we cannot be certain of the significance of the elements in question.

I think that all the facts, so far as they can be determined from the specimen, bearing on the course of the leaf-traces, have now been stated. The evidence is not complete, but the following conclusions may be drawn: A single strand is given off from the stele. In traversing the cortex it

divides up, in a manner not determined. Before entering the base of a bract, four strands range themselves in a row and constitute the vascular supply of that bract. The four bundles then unite into two (sometimes with an intermediate stage of three), and on entering the bract the two fuse to constitute the great median bundle of the organ. From this main bundle lateral strands are immediately given off which may undergo further branching.

All the leaf-trace and foliar strands observed have a stelar structure. Thus in every respect the vascular supply of the foliar organs is remarkable, and unlike that in other plants of the period.

NOMENCLATURE AND DIAGNOSIS.

As will be seen from the title of the paper, I propose to create a new and purely non-committal generic name, *Cladites*, which may probably prove useful for other undetermined specimens besides the one here described. Just as *Conites* and *Phyllites* denote cones or leaves of unknown affinity, so is *Cladites* intended to serve the same purpose for fossil shoots of like obscurity. It is surprising that no such name should have been thought of before. We may define our provisional genus as follows:

Cladites, gen. nov. A leafy branch or shoot of a fossil plant of undetermined affinity.

As the first specimen described under this name happens to be a petrified fossil, showing structure, it may be thought desirable to limit the genus in future to specimens in a similar state of preservation. This point, however, I will leave to others.

I have named our species *bracteatus* because the foliar organs appear from their structure not to be vegetative leaves, but more probably of the nature of bracts. They might be scale-leaves of some other kind, but the name will serve. The following specific diagnosis may suffice to identify the fossil:

Cladites bracteatus, sp. nov. Shoot stout, in comparison with the dimensions of the leaves. Leaves crowded, simple, probably of the nature of bracts.

Stele medullate. Wood dense, apparently all secondary. Central tissue consisting in part of barred cells.

Mesophyll of bracts undifferentiated. Vascular system consisting of a very large elliptical median bundle with small lateral branches. All foliar bundles with a concentric, stele-like structure, and secondary wood.

Locality. Shore, Littleborough, Lancashire.

Horizon. Lower Coal-measures. From a roof-nodule.

DISCUSSION.

We must now consider the possible affinities of our fossil, even though no definite conclusion can be reached. Certain groups can be excluded at once. Thus we may dismiss the Articulatae as having nothing in common with our *Cladites*.

The Lycopods, however, have to be considered. The somewhat microphyllous habit, with crowded leaves, is not unlike. The secondary wood in the leaf finds a remote parallel in *Sigillaria*, where this structure occurs in some species in the leaf-base, though in the lamina its place is taken by transfusion tissue. Otherwise there is no resemblance in the leaf-structure. The crenulated margins of the wood in the axis might recall Sigillarian structure, but the analogy is deceptive. In *Sigillaria* the crenulations depend on the outline of the primary xylem. In our fossil there is no primary xylem comparable to that of a Lycopod. The barred elements of the central tissue are short and quite unlike the tracheides of a normal xylem. Whatever the homologies of this zone may be, it is clearly so much modified that no comparison with Lycopod structure is admissible. We have already seen (p. 338) that an analogy with the structure of *Lepidodendron selaginoides* is too remote to be significant. The dense, small-celled secondary wood is also very different from that of the Carboniferous Lycopods. The absence of tangential pitting is another important difference, for in fossil Lycopods the scalariform sculpturing extends to all walls of the secondary tracheides. On the whole, it appears that any idea of an affinity to the Lycopodiales must be rejected.

The ferns are manifestly out of the question. Our fossil has relatively small leaves and possesses no true primary xylem. The same objections apply to the Pteridosperms.

Of all known contemporary plants the Cordaitales are the group to which our fossil would most naturally be referred. We must, therefore, inquire how far such a reference appears justified. First of all we may exclude the Poroxyleae and the Pityeae on account of the entire absence of centripetal wood or medullary bundles in the axis of our *Cladites*. Thus it is only the family Cordaiteae which has to be seriously considered. The external habit might well be that of a small Cordaitean shoot. The dense wood, entirely centrifugal and with the elements in regular radial series, is essentially what we find in the genus *Cordaites*, as investigated by Renault. The small rays agree quite well, but the pitting, so far as observed, is different. In our fossil it appears to be scalariform, and no tracheides with multiseriate bordered pits were detected. The preservation, however, is unfavourable for the observation of details. It must also be borne in mind that in the fertile shoots of certain Cordaiteae, e.g. *Mesoxylon multirame*, only spiral and scalariform tracheides are present (6), (8). It is quite

possible that our specimen may have been the base of a fertile shoot of some kind, so the apparent absence of pitted tracheides is not a strong argument against the reference to Cordaiteae.

The bracts, of course, bear no resemblance in structure to the well-known leaves of Cordaiteae. But as they are certainly not normal, vegetative leaves, a different structure is only to be expected. We might better compare them with the bracts of *Cordaianthus* (1), (7). These belong to the male or female catkins. Each bract is traversed by a single median bundle, which Bertrand described as identical with a single nerve from the upper part of the vegetative leaf of *Cordaites* (l. c. p. 34). This is not at all like the much more robust vascular system of our *Cladites* bracts, but the scale is very different, the *Cordaianthus* appendages being so much the smaller.

The remarkable course of the leaf-trace in our fossil, starting as a single strand, then dividing up, next fusing again into a single strand before branching out once more on entering the bract, is quite peculiar, and I know of no analogy among Cordaiteae.

The stout shoot of the *Cladites*, densely clothed with bracts, is totally unlike the naked and commonly slender stalk of the *Cordaianthus* fructifications. It must, however, be remembered that there are several genera referable or allied to the Cordaiteae of which the fertile shoots are so far unknown. On the whole, we may conclude that the organization of our fossil, while not inconsistent with Cordaitean affinities, by no means affords convincing evidence of such a relationship.

One further possibility remains to be considered. Can it be that our fossil belonged to some early representative of the Coniferae? This may seem highly improbable, for no Conifers as old as the Lower Coal-measures are known. Dr. Florin, of Stockholm, whom I consulted on this point, informs me that the earliest known remains of the coniferous genus *Walchia* are referred by Bohemian geologists to the uppermost Westphalian. Otherwise Conifers have not been recorded from older rocks than the Stephanian (Upper Coal-measures). There is thus a considerable gap between the earliest Conifers hitherto recorded and our Lanarkian fossil. Still it is clear that the coniferous phylum must have started further back than our present records show.

The habit of our specimen might quite well be that of a coniferous shoot. In fact this resemblance struck me from the first, and under the influence, no doubt, of the prevalent prejudice in favour of Araucarian antiquity, I have always, in my notes, called the specimen an *Araucarites*. But the evidence is so slender that I could not publish it under that name, and chose the indifferent *Cladites* to avoid all implications.

The general structure of the centrifugal wood, with no distinct primary xylem, is, as already pointed out, that of a typical conifer. The apparently

scalariform tracheïdes are a difficulty; such a structure, however, is not unknown, at least among fossil Conifers. In the Mesozoic genus *Xenoxylon* the transversely elongated pits may closely simulate scalariform structure. A figure of Dr. Gothan's, showing a radial section of the wood of *Xenoxylon latiporosum*, is just like a corresponding section from our *Cladites* (3). Of course no relationship to *Xenoxylon* is suggested; the comparison is only intended to show that an appearance of scalariform sculpturing is not inconsistent with coniferous affinities.

The tracheide-like structure of the cells of the pith or central tissue is a peculiar feature of our fossil. I have already compared it with the similar conditions in the genus *Bilignea*, a remote comparison certainly not indicating affinity. A further remote analogy may be found among Conifers in the parenchymatous tracheïdes discovered by Rothert in the pith of species of *Cephalotaxus* (4).

The most remarkable character of the *Cladites* is the vascular structure of the bracts and the behaviour of the leaf-traces. A somewhat striking, though only partial, analogy is afforded by the cone-scales of *Araucaria imbricata* as described by Seward and Ford (10). At a point just beyond the base of the nucellus they find that the scale 'contains, in addition to the lower series of small collateral bundles, two large concentric strands and a median strand of similar structure.' 'The three concentric strands gradually approach one another, and finally coalesce to form the elliptical strand seen in Fig. 27, H.' The two figures (Fig. 27, G and H) are remarkably like sections of a *Cladites* bract before and after fusion of the concentric strands to form the main bundle. The analogy is only partial, first, because the lower series of small collateral bundles is absent in our case, and secondly, because Seward and Ford's description relates to a fertile cone-scale, complicated by the presence of an ovule and ligule. The authors, however, state that 'the behaviour of the vascular strands in a sterile cone-scale is almost identical with that already described' (l. c.).

Professor Seward kindly had a number of sections of the cone axis and scales of various species of *Araucaria* prepared for me by his assistant Mr. Scott. In a section of a sterile scale of *A. imbricata* I observed a remarkable fusion of bundles, but less regular than in the case figured. I may add that the sections of the cone-axes showed no close analogy with our fossil, the secondary wood in the recent plant being much less developed. A single leaf-trace divides up, as in our case; occasionally, as we have seen, a partial re-fusion occurs, accompanied by concentric structure of the strands. I give the comparison for what it is worth. It is interesting, but probably of little value as evidence of affinity.

To sum up: the available data are insufficient to determine the systematic position of our fossil. I do not believe that it was a Pteridophyte. The Lycopods are the only known Cryptogamic group with which it

could be compared, and the comparison, as we have seen, breaks down. The barred medullary cells are at best a poor apology for primary xylem, and I am not inclined to accept it. A Lycopod without primary xylem is not known to exist.

The whole organization indicates a Gymnosperm of some kind. I think the probabilities are fairly evenly balanced between the Cordaitales and early Coniferae. It is impossible to test adequately the latter hypothesis for so little is known of the anatomy of Palaeozoic Conifers. It is practically certain that they must have existed as early as the Lower Coal-measures, for in the upper beds they were already well developed. Our knowledge of the morphology of Palaeozoic Coniferae is likely to be greatly advanced shortly by the researches, now in progress, of Dr. Rudolf Florin. His short preliminary papers, recently published, already form a useful guide to the subject, one of the most interesting in Palaeobotany (2).

SUMMARY.

The specimen is a petrified shoot from a roof-nodule of the Lower Coal-measures at Shore, Littleborough, Lancs. The following summary is a slight extension of the diagnosis on p. 342.

Shoot stout, closely covered with the simple, bract-like leaves.

Stele medullate, the central tissue consisting in part of cells with tracheide-like sculpturing of their walls, but shaped like the cells of a pith.

Secondary wood dense, with narrow tracheides and low, uniseriate, medullary rays. Pitting apparently scalariform and limited to the radial walls of the tracheides. No distinct primary xylem.

Periderm formed in the outer cortex.

Mesophyll of bracts undifferentiated. Vascular system consisting of a large, elliptical, transversely elongate, median bundle with small lateral branches.

Leaf-trace departing from the stele as a single strand, dividing up in the cortex. About four of the resulting strands supply each bract, fusing into one to form the median bundle from which lateral branches are next given off.

All foliar and leaf-trace strands with a concentric, stele-like structure, and secondary wood.

The possible affinities of the fossil are discussed. It is regarded as a Gymnosperm, allied either to the Cordaitales, or to an early race of Coniferae.

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EXPLANATION OF PLATES XVII AND XVIII.

Illustrating Dr. D. H. Scott's paper on *Cladites bracteatus*.

PLATE XVII.

Fig. 1. Approximately median section of the shoot, showing the wood (as a dark mass) at the top, but passing gradually outwards from above downwards. See p. 340, and cf. Text-fig. 1. (Slide 3). $\times 2$.

Fig. 2. Tangential section (parallel to greater diameter of flattened shoot) passing through the diamond-shaped bases of the bracts. The right-hand side cuts deeper than the left. The foliar bundles are shown at various stages of fusion (Slide 14). \times about 2.

Fig. 3. Transverse section of part of wood, showing four wedges, with some of the internal tissue. Three leaf-trace strands are seen in the disorganized cortex (Slide 9). $\times 16$.

Fig. 4. Tangential section through the wood, showing several of the ligneous wedges. Leaf-traces are seen in the wood at several points. The lower part of the section reaches the central tissue, the upper part the cortex. Cf. Pl. XVIII, Fig. 10 (Slide 15). $\times 4\frac{1}{2}$.

Fig. 5. Tangential section of wood, showing the tracheides and the low, often uniseriate, medullary rays (Slide 15). $\times 42$.

Fig. 6. Approximately radial section of a portion of the wood. The scalariform markings on the radial walls are evident at various points (Slide 3). \times about 90.

PLATE XVIII.

Fig. 7. Tangential section through bracts and outer cortex. In some of the bracts the large median bundle is giving off lateral strands. In the cortex a leaf-trace, at the double stage, is seen; another with much larger bundles is entering a bract (Slide 6). $\times 4\frac{1}{2}$.

Fig. 8. Tangential section through bract-bases. Above, a large leaf-trace, with two strands, is entering a bract. Below, the large median bundle of a bract is fused up, and is giving off a lateral strand on one side (Slide 5). \times about 7.

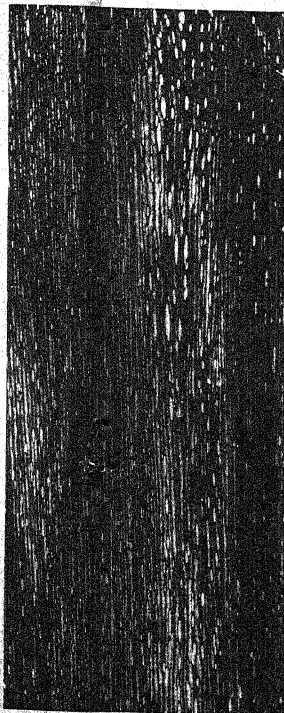
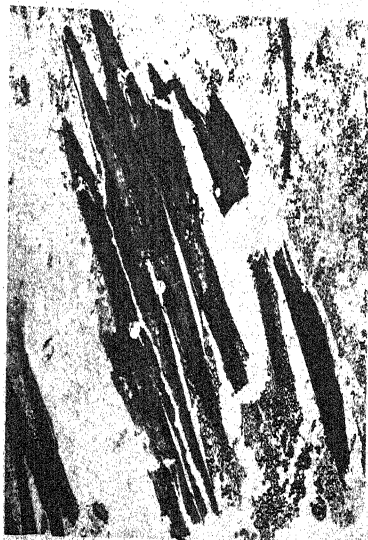
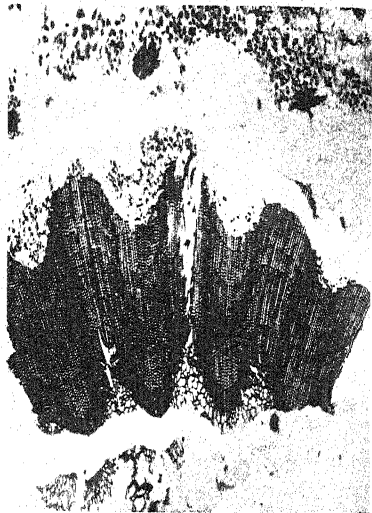
Fig. 9. Lower part of the section shown in Pl. XVII, Fig. 1. Above, a row of four strands is seen, the supply for one bract. Lower down the leaf-trace strands of each bract have fused to form two of increased size (Slide 3). \times about 6.

Fig. 10. Left-hand portion of the section in Pl. XVII, Fig. 4 to show a leaf-trace passing out through the wood. The trace is at the lower edge of the gap in the pointed mass of wood and is cut almost transversely (Slide 15). \times about 20.

Fig. 11. Portion of the transverse section in Pl. XVII, Fig. 3. In the dark masses at the mouth of the gap in the wood some spiral elements can be made out, probably belonging to an outgoing leaf-trace (Slide 9). \times about 110.

Fig. 12. Periderm (*pd.*) in outer cortex of axis, transverse section (Slide 8). \times about 100. [The object was difficult to photograph.]

The photographs reproduced in the plates are the work of Mr. W. Tams, of Cambridge. The drawings for the text-figures were made by Mr. G. T. Gwilliam, F.R.A.S. I am much indebted to both these skilled coadjutors.





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p. d.



W Tans. photo.

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Print. London.

II. SALTATION IN *CYTOSPORINA LUDIBUNDA*.

The species of *Cytosporina* under investigation was originally isolated by Dr. Horne from decayed Lane's Prince Albert apples, held in storage in this Laboratory in 1920, and the same fungus was obtained from fifty different apples. When grown in potato mush agar the fungus produced somewhat irregular stromata which upon maturity discharged slender hook-shaped spores ('B' pycnosporos of Diedicke) of the kind described for the form genus *Cytosporina*. Since the dimensions of the spores agreed very closely with those recorded for *C. ludibunda* in systematic works, the identity of the fungus appeared to be satisfactorily established.

From 1920 onwards this fungus has been utilized in connexion with investigations carried out in this Laboratory, into the internal resistance shown by apples to fungal invasion (15, 21), and showed no marked sign of instability until 1926, when it was found that fungal growths obtained by re-isolation from the diseased tissues present in apples previously inoculated with *C. ludibunda* showed many unusual features. Although 271 re-isolations were made out of 1,500 inoculated apples, none of the growths obtained exactly resembled the fungus originally introduced into the apple. These growths showed great diversity under standard experimental conditions but possessed one character in common, namely the presence of two types of pycnosporos, the 'A' and 'B' pycnosporos of Diedicke (12). Although numerous attempts were made to classify them it was found impossible to do so on an exact basis. They were ultimately and provisionally grouped in seven classes (CR₁–CR₇), each class comprising forms which varied within certain limits. This preliminary work which has only been briefly dealt with here, indicated that the fungus was in a state of 'flux' and led to the detailed investigation of saltation in *C. ludibunda* described below.

Of the four stock cultures of *C. ludibunda* available for experiment, three, C', C'', and C''', showed well-developed stromata and spore-masses. Before using these cultures the spore-masses were thoroughly examined and the spores in every case proved to be of the filamentous type ('B' spores). From these stock cultures eighteen monohyphal cultures were made, using for each the branched or unbranched end of a single hypha. After a few days, fragments of mycelium from the monohyphal cultures were transferred to plates. The growths formed in plate cultures were of a variable nature but could be arranged in four fairly distinct groups (C, C₁, C₂, C₃). The cultures grouped together in C₃ exhibited a combination of the characters of those placed in the three remaining groups. All the cultures produced pycnosporos of the 'A' and 'B' types. The growth characters of C, C₁, and C₂ in standard medium cultures are given below.

C—Aerial mycelium brown, forming a thin felt. Substratum brown. Zonation wide, distinct, or in some cases absent, stromata small, dark brown, scattered, fairly numerous.

C₁—Mycelium superficial, yellowish-brown. Substratum brown. Zonation wide, brown and clear zones alternating. Stromata or pycnidia dark brown, small, restricted to the central region of the culture.

C₂—Mycelium superficial, grey with central band of raised mycelium. Substratum grey. Zonation feeble, wide. Stromata dark brown, few, variable in size.

Three cultures, one from each of the groups C, C₁, and C₂ were selected for more detailed study. The strains were repeatedly subcultured, using mycelial inocula in the standard medium, and the characters shown on each occasion were critically compared. Care was taken to keep the line of descent from each monohyphal parent separate. As a result, in some cases variants were obtained which remained true to type, in others variants which proved to be more or less unstable. In Table I, the relation between the variants derived from the monohyphal parents C, C₁, and C₂ and the groups of the re-isolated strains (CR₁–CR₇) is shown. In each case the positive sign indicates that a particular monohyphal variant falls into one or other of the seven groups. The correspondence between the two sets is remarkable. It is further seen from the table that a strain, CC₂, has been obtained which did not fall into these groups. Similar results were obtained when small masses of pycnospores taken from individual stromata were used instead of mycelium for the inocula.

TABLE I.

Relation between the Monohyphal Variants and the Groups of the Re-isolated Strains of Cytosporina ludibunda.

Strains (Monohyphal).	Nature of the Cultures derived from Monohyphal Strains.							
	CR ₁ .	CR ₂ .	CR ₃ .	CR ₄ .	CR ₅ .	CR ₆ .	CR ₇ .	CC ₂ .
C	+	+	+		+			+
C ₁	+		+	+	+	+		+
C ₂	+	+			+	+		+

The method of plating has, however, yielded the greatest number of variants. Twelve spore-masses taken at random from the monohyphal cultures C, C₁, and C₂ were plated separately, each spore-mass being divided among a number of plates. While the majority of the colonies arising in plates conformed to the parent type, at least four other types were present which were distinctly different. Almost all the plates showed the presence of one or more of these variants, one particular plate, however, prepared

from a spore-mass of C showed all of them. The variants differed in the following respects:

- (a) Drab-white growth with thick mycelium.
- (b) White growth with thin mycelium.
- (c) Grey mycelial growth.
- (d) Dark brown growth with thick mycelium.

The marked dissimilarity of these variants is evident from the photograph of one of the platings given in Pl. XIX, Fig. 1.

From these variants the following strains were obtained:

CA—derived from variants (a) and (b). Thin drab-white mycelial felt with moderately numerous stromata of variable size. Zonation wide, (Pl. XIX, Fig. 2.)

CB—derived from variant (c). Dusky brown, showing distinct zonation. This strain proved to be quite sterile.

CC—derived from variant (d). Thin brown mycelial felt and dark grey to grey substratum. Large stromata disposed in zones. Zonation wide. (Pl. XIX, Fig. 3).

The plating experiment was repeated several times but only one additional strain was obtained—namely CC₂ (Pl. XX, Fig. 12), also obtained from mycelium.

Similar experiments were then carried out with the saltant strain CA. Platings of spores were made from time to time. At first the growths obtained were fairly uniform and remained true to type, but later interesting features were observed. Three dilution plates had been prepared, using spores taken from a single spore-head. In one of these plates two variants were obtained which contrasted sharply with the growths representing the parent type, by reason of their colour and relatively small size (see Pl. XIX, Fig. 4). The first variant (a) which showed a dark brown leathery mycelium proved on subculturing somewhat similar to the monohyphal parent C. The second variant (b), which was grey in colour and with few stromata, gave rise to a new strain termed CA₁. CA₁ is characterized by the colour of the mycelium ranging from grey to pinkish vinaceous, wide zonation, the frequent presence of a central band of tufted mycelium, and by the paucity of the stromata (Pl. XIX, Fig. 5).

The strain CA₁ was next subjected to plating experiment. The colonies obtained during the earlier platings were apparently all alike, nevertheless, on various occasions subcultures made from some of the colonies gave rise to a strain CA₂ (Pl. XIX, Fig. 6), indistinguishable, as a rule, from CA₁ in mycelial characters, but differing from it in its inability to form fruit bodies. During the course of later experiments an entirely different variant appeared. It was first observed as a small black colony in sharp contrast with the grey colonies of CA₁; later the contrast became

more pronounced owing to the development of pycnidia where its margin came into contact with the CA_1 colonies. Subcultures from the black colony showed a black mycelium and substratum, in which as time went on innumerable small fruit bodies were formed, generally disposed in zones. Several plates were made, using mycelial inocula from the black sporing cultures, the resulting growths showing the presence of grey and black mycelium side by side, and from which eventually two very distinct strains were separated, namely CA_4 (Pl. XIX, Fig. 8), an infertile black strain, and CA_3 (Pl. XIX, Fig. 7), a fertile strain, smoke-grey in colour, differing from CA_1 mainly in its more prolific sporing.

Platings from the spores of CA_3 yielded besides the parent, colonies of CA_1 and CA_2 , both grey, and colonies of CA_4 the black strain (Pl. XIX, Fig. 9). The production of the black strain by CA_3 proved to be of particular interest, and has been separately dealt with in p. 356.

Attention was then re-directed to the second sporing strain CC, originally derived from the monohyphal parent C.

Series of plates were made, using different spore-heads, and, in every plate but one, brown colonies showing the character of the parent were obtained. In the remaining case, besides the parent colony CC, two variants were present, showing (a) light amber-brown, and (b) pale orange-yellow growths respectively (Pl. XIX, Fig. 10). Upon subculturing, both of these colonies yielded the same saltant (CC_1), which is light amber-brown in colour with numerous small pycnidia generally distributed throughout the culture (Pl. XX, Fig. 11), in contrast to CC which shows dark grey mycelium and large stromata in zones (Pl. XIX, Fig. 3). Further, while CC produced both 'A' and 'B' spores, the saltant CC_1 produced only the 'B' type. The platings from the spore-heads of CC_1 yielded only a few colonies of two slightly different types, both of which were pale orange-yellow in colour, but whereas in one of the types the colour was uniform, the other showed an amber-brown marginal zone. Both on subculturing produced the same saltant, which appeared to be completely sterile and identical with CC_2 (Pl. XX, Fig. 12). This strain was also obtained by direct plating from the monohyphal parent C.

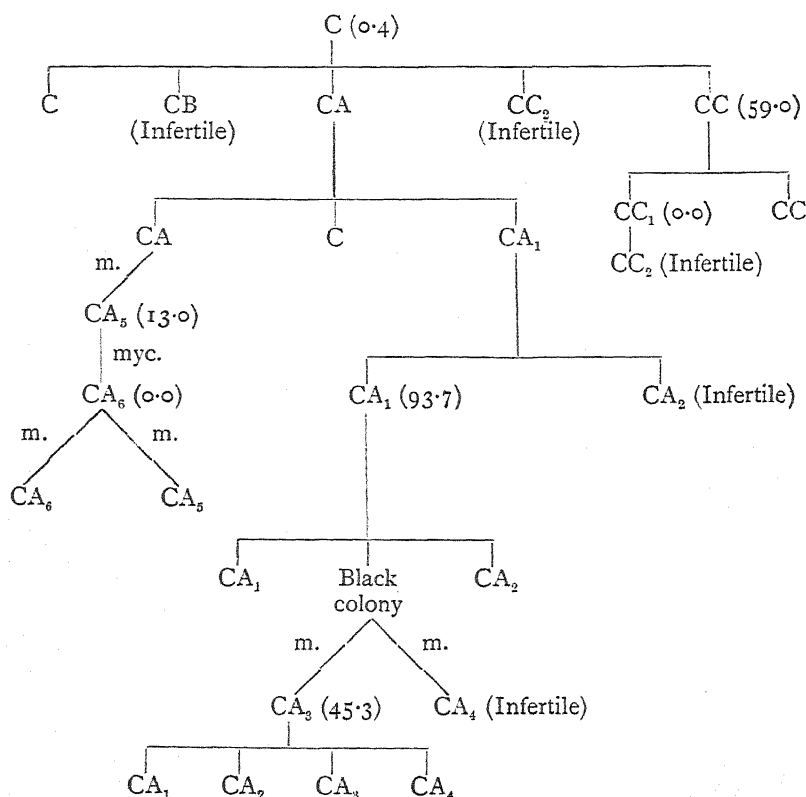
By this method of plating no less than nine distinct strains had been obtained which were derived from a single culture of monohyphal origin. The origin of these saltants is shown diagrammatically in Text-fig. 1.

In order to test the constancy of these saltant strains, further cultural work was carried out using only the mycelial inocula. The result is briefly dealt with below:

CA—Constant for a number of cultural generations, subsequently gave rise to an 'ever-saltating' strain termed CA_5 (Pl. XX, Fig. 13), from a part of the culture where no sectoring was evident. CA_5 will be described in greater detail below.

CB—After three cultural generations reverted to a variant type already obtained.

CC—Variation was observed from time to time. The colour varied from dark brown to grey; the stromata were always large but varied greatly in number.



TEXT-FIG. 1. Diagram showing the origin of certain strains of *Cytosporina ludibunda* from a single monohyphal parent.

The origin of strains is from spores except where mycelium (m) is indicated.

Figures against the strains show the average percentage of 'A' spores present in each case.

CA₁—This fertile strain usually bred true to type. Occasionally, however, it produced the sterile saltant CA₂ though no sectoring could be observed in the culture.

CA₂—This infertile strain remained true to type.

CA₃—This strain usually bred true to type. Occasionally sectoring cultures were obtained, the sector showing the characters of CA₂ (Pl. XIX, Fig. 7). CA₂ was also isolated from regions where no sectoring was evident.

An interesting phenomenon was observed while plating the spores of

CA₃ which was not met with during the mycelial subcultures. It has been described in some detail on p. 356.

CA₄—This infertile strain remained true to type.

CC₁—Remained true to type for a few generations, and then it was found that from whatever part of the mycelium the subcultures were made, infertile growths were obtained which resembled CC₂.

CC₂—This apparently infertile strain proved somewhat variable in colour. In exceptional cases subcultures were obtained in which pycnidia with 'B' spores were formed, in this respect resembling CC₁, but differing from the latter in all other characters.

It will be seen that only one new strain CA₅, was obtained in the course of the tests mentioned above. This strain proved to be particularly interesting and therefore deserves more detailed consideration.

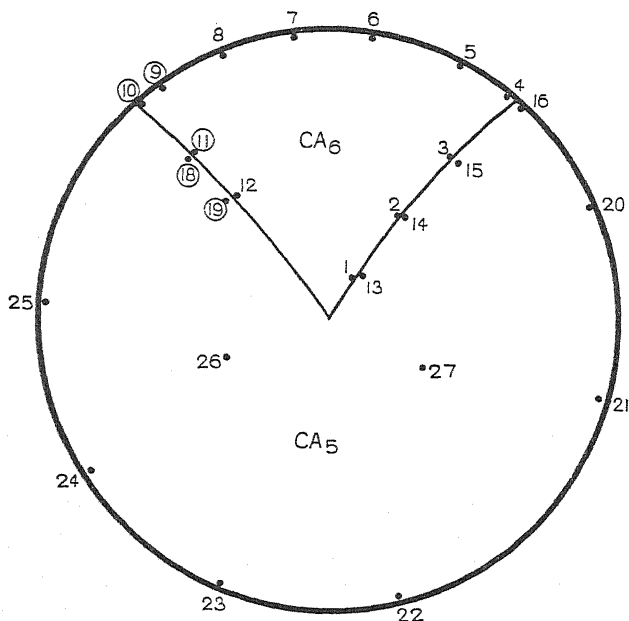
CA₅ differs from CA in producing fewer stromata and in its 'ever-saltating' character. In almost every subculture CA₅ produces sectors, generally one, sometimes more than one, which are quite unlike the parent. In CA₅ the colour of the mycelium is drab-white, and stromata with 'A' and 'B' spores are present; in the sector (CA₆), on the other hand, the colour is brown and numerous small pycnidia with 'B' spores are formed (Pl. XX, Fig. 13). Several preliminary attempts were made to isolate the saltant representing the sector, but without success. The sectoring cultures were then studied in a more systematic manner. A sectoring plate culture of CA₅ grown at room temperature was chosen, and a number of mycelial inocula were taken from different regions, namely from the youngest portion of the culture and also from the regions lying on either side of the line demarcating the saltant from the parent. The particular places from which the inocula were taken is shown in Text-fig. 2, the numbers referring to particular inocula. Each inoculum was transferred to a separate standard medium plate, and these were kept under the experimental conditions employed for the parent cultures. As a result it was found that the growths originating from inocula 9, 10, 11, 18, 19, showed the characteristics of CA₆, the remainder proved to be a mixture of CA₅ and CA₆ in various proportions.

The pure saltant CA₆ was obtained from both the sector (CA₆) and the parental region of the culture (CA₅). It was obtained from both young (Nos. 9, 10) and old (No. 11) portions of the sector, and from old portions (Nos. 18, 19) of the parental region, but it was never obtained from the youngest parental mycelium.

The experiment was then repeated, using the sectoring culture of CA₅ arising from inoculum No. 20, and the pure saltant culture (CA₆) obtained from inoculum No. 9. The results, in both cases, were similar to those obtained in the first experiment. When cultures of CA₅ and CA₆ selected from the second series of plates were in turn subjected to similar experimental

treatment, it was found that none of the inocula, whether taken from CA_5 or CA_6 , produced the pure saltant CA_6 .

It will be at once evident from the results described above that CA_6 usually does not breed true to type but shows reversion to parental characters.



TEXT-FIG. 2. Diagrammatic representation of sectoring culture CA_6 showing positions 1-27 from which the inocula were taken. The numbers within the circle indicate the positions from which the saltant CA_6 was obtained. No. 17 has been accidentally omitted; its position was in CA_5 close to No. 10.

It is interesting to note that at higher temperatures, viz. 25°C . and 30°C . sectoring was not observed in CA_4 . This strain is still under investigation.

During the course of the spore plating already described (see p. 353), it was found that the relative proportion of grey (CA_1 , CA_2 , CA_3) and black (CA_4) colonies derived from the spores plated from individual stromata of the strain CA_3 varied within narrow limits. In platings made from the first cultural generation of CA_3 , the percentage of black colonies varied from 4.1 to 8.0, while the average percentage for the stromata examined was 5.95. When a similar experiment was made with the same strain, using the third cultural generation (CA_{3-3}) arising from spores, not only did the proportion vary within wider limits, but also the average percentage of black colonies was much higher (38.9), and it was also higher for all the individual stromata examined (13.6 to 63.4). The results are given in detail in Table II.

TABLE II.

Percentage of Black Colonies derived from Different Stromata of CA₃.

CA₃-(1). First Cultural Generation.

Stromata.	Percentage of Black Colonies in each Plate.					Average percentage of Black Colonies.
	1	2	3	4	5	
1	10.5	10.0	7.5	6.0	5.0	8.0
2	10.0	9.0	7.5	3.5	4.0	6.8
3	7.5	5.5	3.5	3.0	—	4.9
4	7.0	4.0	3.5	3.0	3	4.1

CA₃-(2). Third Cultural Generation.

1	69.5	63.0	60.0	61.0	—	63.4
2	52.0	49.0	48.5	47.0	46.5	48.6
3	50.0	49.5	39.5	—	—	44.6
4	40.0	39.5	—	—	—	39.3
5	44.5	37.5	36.0	34.5	32.5	37.0
6	44.5	37.5	30.5	23.5	—	34.0
7	33.5	32.5	32.0	31.0	23.0	30.4
8	17.0	16.5	13.5	13.5	7.5	13.6

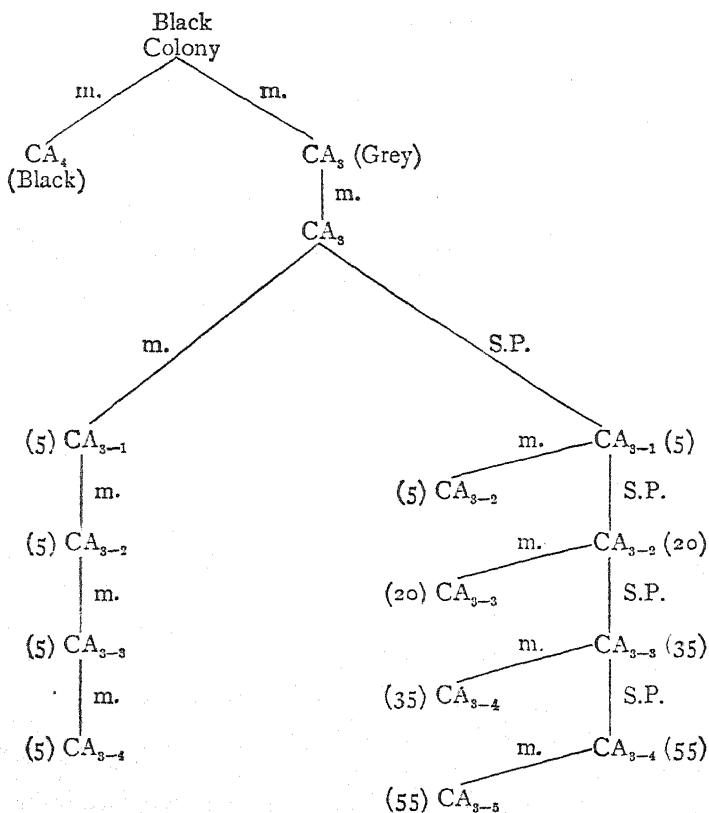
For a further elucidation of the point, investigations were carried out in greater detail by plating spores from different spore-heads and calculating the proportion of 'Blacks' and 'Greys' in each case. During plating every care was taken to standardize the experimental conditions.

The first dilution plate cultures made from different spore-heads of the original culture of CA₃ produced colonies the majority of which were grey (CA₁, CA₂, and CA₃), a few being infertile black (CA₄). The average percentage of 'Blacks' for this particular plating experiment was 4.5. The colonies arising from the first plating were then allowed to grow until some of the CA₃ colonies had sporulated. Platings were then made from a number of spore-heads of one of these colonies (2nd plating). The result was the similar appearance of 'Blacks' and 'Greys'. On this occasion the average percentage of 'Blacks' increased to twenty. Platings were again made, using spore-heads from a colony of CA₃ obtained from the 2nd plating. The average percentage of 'Blacks' in this instance was still higher, viz. 35. Similar platings were made for a few more generations, always using spore-heads from one of the CA₃ colonies obtained as a result of the previous plating. It was found that in the 4th plating generation the percentage of the 'Blacks' had reached its highest, viz. 55, beyond which no further definite increase was observed.

Experiments were made to determine whether the occurrence of black colonies was related to the time of development of spores. Platings were made using the first spores of a stroma, and some weeks later further platings were made from the later discharge of the same stroma. In both cases a certain percentage of black colonies was obtained.

In order to determine whether similar changes in the relative proportion of 'Greys' and 'Blacks' occurred, when the inocula used were of mycelial nature, subcultures were made from some of the CA_3 derivatives obtained from pycnospores using mycelial inocula. When spore-discharge was observed in these cultures they were plated in the usual way. As a result it was found that the cultures developing from mycelial inocula showed approximately the same proportions of 'Blacks' and 'Greys' as those recorded for the parent cultures from which they were derived. For example, the culture derived from a colony which produced 5 per cent. 'Blacks' also produced 5 per cent. 'Blacks', that derived from one producing 20 per cent. 'Blacks' also produced 20 per cent. 'Blacks' and so on.

The descent of CA_3 through mycelium and pycnospore respectively, and the percentage of 'Blacks' shown by various descendants are represented diagrammatically in Text-fig. 3.



TEXT-FIG. 3. Diagrammatic representation of the descent of CA_3 through mycelium and pycnospores respectively. The approximate percentage of black colonies shown by various descendants is given in brackets. m. = mycelium; S.P. = spore plated.

III. CHARACTERISTICS OF THE SALTANTS (Standard Medium Cultures).

Since the majority of the strains from the stock culture of *Cystosporina ludibunda* proved to be unstable, a detailed description of all the variants would serve no useful purpose. It is proposed instead to give, first of all, a short general account of the more important characters shown by the variants, and then to compare the characteristics of some selected saltants which have been specially studied in the paper, devoting special attention to spore characteristics, concerning which very little information has been given in the preceding pages.

The general morphological characters of the variants as observed in the standard medium cultures were as follows :

Colour of the mycelium and substratum. Pure white to various shades of grey, yellow, brown, and black: cultures show one or more of these colours.

Nature of the mycelium. Aerial mycelium may be present or absent. When present forming a shallow or moderately dense felt, in some cases loose, more or less scattered tufts are present. Some strains show torch-like outgrowths, these may be erect or depressed and variously coloured, namely, white, white and yellow, yellow and brown, &c. Zonation may be distinct, indistinct, or absent; the zones may be wide or narrow.

Asexual reproductive organs. All stages between stromata and pycnidia are found, and one or other or both may be produced by the same strain. Stromata are subspherical or irregular, smooth or covered with a mycelial felt, and show various colours, namely, shades of grey, brown, and black. Stromata and pycnidia are few to extremely numerous, scattered or disposed in zones. Spores are discharged in cream, pink, or yellow spherical masses, or in similarly coloured tendrils. The same strain may show both kinds of spore discharge. The spores are of 'A' and 'B' types.

For the sake of convenience the comparison of the selected saltants is given below in tabular form (Table III).

It will be seen that the difference between CA_1 , CA_2 , and CA_3 , lies only in the degree of fertility. Again, the strains CA_6 and CC_1 , although very similar in morphological characters, have been considered as distinct for some reasons. Thus CA_6 on subculture almost always reverts to CA_5 , whereas CC_1 on subculture gives rise to CC_2 , the products in the two cases being entirely different.

The sporling saltants with two exceptions produce both 'A' and 'B' spores, the exceptional strains producing only the 'B' type. The characteristics of these two types of spores are given below :

'A' spores. Hyaline, short, sub-elliptical, sub-ovoid, or sub-oblong. Usually with two distinct oil-drops, sometimes more than two; exceptionally oil-drops are absent (Text-figs. 5, 6, 7, 8).

TABLE III.

Characters of Some Selected Strains in Standard Medium Cultures.

Sal- tants.	Colour.	Sub- stratum.	Mycelial Char- acter.	Zona- tion.	Repro- ductive Char- acter.	Nature of Repro- ductive Organs.	Spores.
CA	Drab- white.	Grey.	Thin felt.	Wide.	Fertile	Stromata : dark brown, variable in size. Fairly numerous, disposed in zones.	'A' and 'B'
CC	Brown	Brown.	Thin felt.	Wide.	Fertile	Stromata : dark brown, usually large disposed in zones.	'A' and 'B'
CA ₁	Grey to pinkish-vina- ceous.	Grey.	Thick felt, with a central band of tufted mycelium.	Wide.	Fertile.	Stromata : dark brown, variable in size. Very few.	'A' and 'B'
CA ₂	Do.	Do.	Do.	Do.	Infertile.	—	—
CA ₃	Do.	Do.	Do.	Do.	Fertile.	Stromata : dark brown, variable in size. Numerous, disposed in zones.	'A' and 'B'
CA ₄	Black.	Black.	Thick felt, some- times fluffy.	Absent.	Infertile	—	—
CA ₅	Drab- white.	Grey.	Thin felt, 'ever- saltating'.	Wide.	Fertile.	Stromata : brown, very few ; some- what large.	'A' and 'B'
CA ₆	Brown.	Brown.	Thin felt	Narrow.	Fertile.	Pycnidia : brown, numerous, small, scattered.	'B'
CC ₁	Light amber- brown.	Do.	Do.	Do.	Do.	Do.	Do.
CC ₂	Pale orange- yellow.	Do.	Do.	Do.	Infertile.	—	—

'B' spores. Hyaline, filiform, straight or bent. All intermediate forms are found between straight and strongly bent spores. The spores of different strains vary with regard to the average degree of bending. They are not of uniform width, the straight end being slightly wider ; the bent end is more or less pointed (Text-figs. 5, 6, 7, 8).

Taking all the strains into account the length of the 'B' spores varies from 18–40 μ ; the variation in width is comparatively small, viz. 1–2 μ . The 'A' spores are shorter, varying in length from 4–16 μ , and in the mean length from 9–10 μ . The variation in width (2.5–5 μ) is greater than that

shown by the 'B' spores. With regard to the difference between strain and strain in spore length, some strains vary within relatively narrow limits, with others the range is wider. As to the mean length the strains show all gradations between the extremes given. These features are shown more markedly by the 'B' spores. In Table IV the limiting and mean lengths in μ for the 'A' and 'B' spores of certain selected strains are given, arranging the strains in order of increasing spore-length.

TABLE IV.

Length of 'A' and 'B' Spores in Certain Selected Strains.

Strain.	'B Spores'.		'A Spores'.	
	Range in μ .	Mean in μ .	Range in μ .	Mean in μ .
CC	21-32	26	4-12	9
CA ₆	20-32	29	—	—
C	24-36	30	6-16	9.5
CC ₁	22-38	30	—	—
CA ₃	18-40	32	6-12	9.5
CA ₅	21-40	34	8-14	10

The following points are at once evident: (1) The gradation in length mentioned above; (2) an increase in the mean length of 'A' spores is associated with an increase in the length of 'B' spores; (3) the variation in the mean length of 'A' spores from strain to strain is slight, although in each case the variation covers a wide range.

Brefeld (2) and Wehmeyer (29) found a certain relation between the colour of the spore-mass and the kind of spores in certain species of *Diaporthe*. Thus pink or creamy spore-horns were made almost entirely of 'A' spores; yellowish or white spore-horns, on the other hand, contained usually spores of the 'B' type. A similar correspondence in colour and spore-type is shown by the strains derived from *Cytosporina ludibunda*. Cream or yellow spore-masses or tendrils consist almost entirely of 'A' spores, whereas the white ones are almost exclusively 'B' in character.

With regard to the appearance of 'A' and 'B' spores, Brefeld (2) (*Diaporthe inaequalis* Curr. and *D. spina* Fkl.) and Harter (18) (*D. phaseolorum*) found that the 'A' spores make their appearance in the stroma first, the 'B' spores later. On the other hand, Wehmeyer (29) (*D. oncostoma*), Archer (1) (*Phomopsis arctii*), Cayley (9) (*P. perniciosa*), and Kidd and Beaumont (23) (*P. mali*) record that the 'B' spores develop first, the 'A' spores later. The writer's observations were confined to the periodic examination of the spore-heads of certain *Cytosporina* derivative strains, selecting for the purpose those which produced both 'A' and 'B' spores. Some time prior to the discharge of spores a drop of water is exuded at the tip of the ostiole of the stroma, into which the spores are subsequently discharged.

Liquid was taken from the drop from time to time, using a very fine capillary tube, and examined microscopically.

This method, of course, indicates only the order of the discharge of spores; however, it is thought not unlikely that a certain relation exists between the order of development and discharge. It was found that no general rule could be laid down as to the order of appearance of 'A' and 'B' spores. The stromata discharging 'A' spores may sooner or later discharge 'B' spores in addition and vice versa, so that the spore-heads ultimately contain a mixture of 'A' and 'B' spores. Sometimes, however, some of the stromata producing only one kind of spores may not produce the other kind at all, so that a stroma with pure 'A' or pure 'B' spores results.

The 'A' and 'B' spores are present in different proportions in the stromata or pycnidia. With some strains, for example, CA₁ and C, the two kinds of spores are formed nearly in the same proportions in all the stromata in a given culture, with others (CA₅, CA₃, CC) the ratio varies. The variation reaches an extreme in CC, where stromata containing 'A' spores only, various proportions of 'A' and 'B' spores, and 'B' spores only, are found in the same culture. In standard medium culture, the strains themselves show almost every gradation between the extreme types (1) with 'B' spores only, (2) with 'A' spores only. The point is clearly shown in Table V, where the range of variation in the percentage and the mean percentage of 'A' spores shown by certain stromata are given for various selected strains. In each case the number of stromata examined and the number of spores under observation are stated.

TABLE V.

Occurrence of 'A' Spores in Certain Selected Strains.

Strain.	Number of Stromata.	Number of Spores.	Variation in Percentage.	Average. Percentage.
CC ₁	20	20,000	0	0.0
C	8	40,166	0-5	0.4
CA ₅	8	3,900	0-21	13.0
CA ₃	15	13,712	0-59	45.3
CC	10	8,289	1.6-100	59.0
CA ₁	5	9,000	90-95.6	93.7

Cayley (9) has recorded that in *Diaporthe perniciosa* the 'B' spores disintegrate with age, leaving only 'A' spores in the stroma. No definite evidence of such disintegration of 'B' spores has been observed in the case of *Cytosporina*. In some strains the 'A' spores undergo a certain amount of disintegration, as is evident from their size and shape.

IV. VARIATION IN GENERAL MORPHOLOGICAL CHARACTERS AS OBSERVED IN DIFFERENT NUTRIENT MEDIA.

These experiments were undertaken to find out to what extent the general morphological characters shown by any given strain varied when different nutritive media were employed. For example, whether in certain media stromata were formed, in others pycnidia, or in certain media spores of one type only, in others both 'A' and 'B' types.

The media were chosen to show some marked differences from the standard medium in one or other of the constituents, i.e. the sugar contents, nitrogen contents, &c. The composition of the media selected is given below for reference, each constituent being given in grammes per litre of water.

TABLE VI.

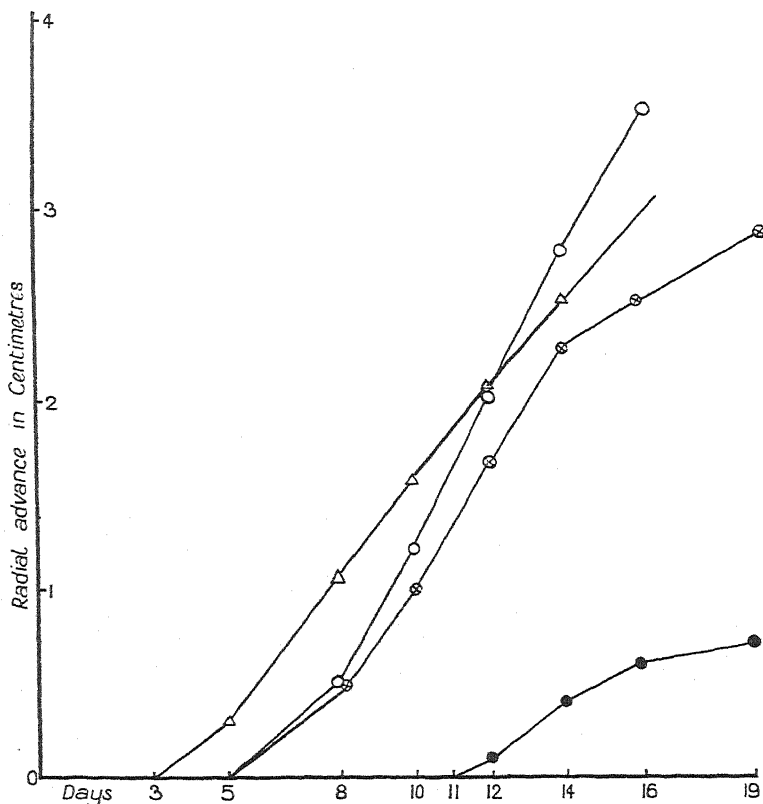
	Standard.	Coons'.	Malt.	Richards'.
KNO ₃	—	2.00	—	10.00
MgSO ₄	0.75	1.20	—	0.25
KH ₂ PO ₄	—	2.70	—	5.00
K ₃ PO ₄	1.25	—	—	—
FeCl ₂	—	—	—	trace
Asparagin	2.00	—	—	—
Cane sugar	—	—	—	50.00
Maltose	—	7.20	—	—
Glucose	2.00	—	—	—
Malt extract (commercial)	—	—	20.00	—
Potato starch	10.00	10.00	10.00	10.00
Agar	15.00	15.00	15.00	20.00

In Coons' and in malt extract agar the strains utilized usually show distinctive characters, but in Richards' medium the growths formed by certain strains so closely resemble one another that it was difficult to distinguish between strain and strain. As a rule the cultures in Coons' medium do not markedly differ from those obtained in standard medium. The zonation is, however, less marked, and the colour more strongly developed. The strain CC₂ proved to be exceptional, the characters of the cultures being entirely changed, showing superficial mycelium without zonation and dark green colour instead of the mycelial zones and orange-yellow colour characteristic of standard medium culture. Cultures of CC₂ grown in the two media differed to such an extent that they might easily be mistaken for different fungi.

Malt agar cultures usually show more aerial mycelium than that developed in Coons', and the zonation is indistinct. The colouring is more strongly developed than in the standard medium. The colour shown by CC₂ varies from dark grey to dark green.

Growths in Richards' medium usually show an irregular outline, and the aerial mycelium forms a thick compact felt. Colour of the mycelium

is at first white or almost white, subsequently changing into brown; ultimately the aerial mycelium becomes tough and wrinkled, forming a crust on the surface of the medium. The strain CA_4 alone retains its main



TEXT-FIG. 4. Graph showing the rate of radial advance in cm. of the strain CC_2 for four different media at room temperature.

Δ = standard synthetic medium.

⊗ = malt agar.

○ = Coons' agar.

● = Richards' agar.

characters observed in the standard medium cultures. C , CC , and CA_5 are indistinguishable from one another. CA_2 and CA_3 resemble one another, and differ only slightly from C , CC , and CA_5 . In the case of CC_2 the radial growth is greatly retarded, but the surface of the culture is covered with dense white tufts of aerial mycelium. This point is illustrated in Text-fig. 4, where the rate of radial growth in centimetres is given for four different media.

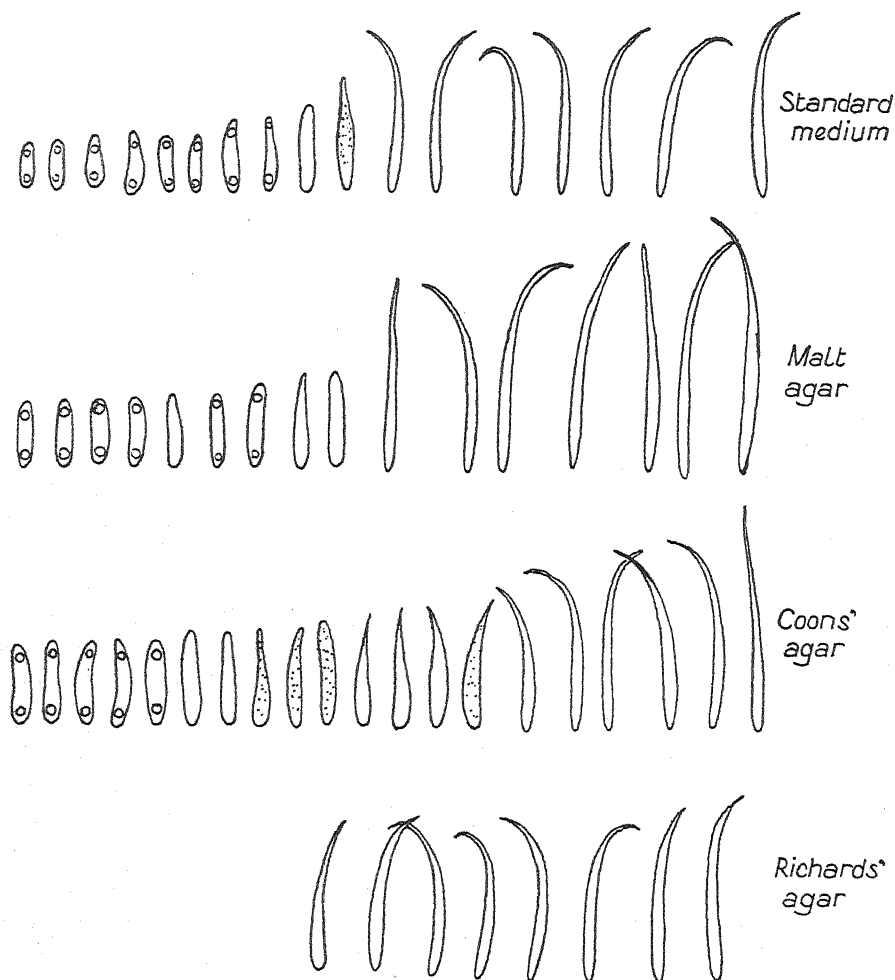
The change of medium was reflected in a very striking manner in the reproductive characters. Strains which proved infertile in the standard medium were also infertile in Richards' medium. In the case of sporing strains it was found that instead of the large stromata formed by certain strains in the standard medium, the same strains in Richards' medium

produced only relatively small stromata or pycnidia. Among the strains used for these experiments there were four, viz. C, CA₃, CA₅, and CC, which in standard medium cultures invariably produced 'A' and 'B' spores in varying proportions. All these strains in Richards' medium *produced only 'B' spores*. In each case, culture in one medium conformed in sporing character to the form genus *Phomopsis*; in the other medium to the form genus *Cytosporina*. In some of these media, again, the spores showed a great variability in their morphological characters which is worthy of special consideration.

Van Höhnelt (28) and Bubák (7, 8) found all transitional stages between 'A' and 'B' spores in some species of *Phomopsis*. Diedicke (12), on the other hand, in 'Die Gattung *Phomopsis*', denied the occurrence of such intergradation in any of the species investigated by him. Later on, finding intergradation in *P. arctii*, he stated (13) that the culture was 'abnormal'. The observations of these authors were confined to the behaviour of the fungi growing under natural conditions. That intermediate stages are produced in artificial cultural media has been noted by Brefeld (3) for some species of *Diaporthe*. The observed variability in the dimensions of the spores shown by certain strains in culture suggested the occurrence of intergradation in *Cytosporina* also, and the question was followed in some detail. The four strains C, CA₃, CA₅, and CC were cultured in the four media already mentioned. When the cultures, sixteen in all, were in a sporing condition, microscopical examination of all the cultures was made, choosing spores taken at random from various stromata or pycnidia. Next, slides were prepared to represent quite fairly the nature of the sporing shown by each strain in each of the media used. Finally a representative 'field' was selected in each case, and the spores were measured and drawn. The result is illustrated in Text-figs. 5-8, the spores in each case have been arranged in serial order of length.

It will be seen that a complete intergradation between 'A' and 'B' spores occurs in the strain C and CA₅ when grown in Coons' medium (Text-figs. 5, 6). The 'A' spores in the former strain are comparatively large, but in both the strains the smaller 'A' spores contain two oil-drops. In the larger 'A' spores, however, the oil-drops disappear and the spores become either granular or hyaline. The transitional forms are very abundant in C (Text-fig. 5), and gradually taper to a point at one end, but this attenuation is not so prominent in CA₅ (Text-fig. 6). The strain CC shows incomplete intergradation in the standard and malt media, but none at all in Coons' (Text-fig. 7). The intermediate forms are generally of the same type. In CA₃ no intergradation was observed (Text-fig. 8). Grove (16, 17) has observed the occurrence of a third type of spores ('C' spores) in some species of *Phomopsis*. It seems that these spores are in many respects similar to the intermediate spores just mentioned.

Owing to the occurrence of spores which show transitional stages between typical 'A' and 'B' spores, it is clearly difficult to use the



TEXT-FIG. 5. Illustrates the spore characters of the strain C in various media.

character of spore dimensions for comparative purposes. The difference between strain and strain is often more adequately expressed by the extent to which the spores vary within a given range of experimental conditions. The question of spore dimensions will therefore be considered very briefly here. In Table VII the limiting range in length and width in μ for the 'A' and 'B' spores only is given for the strains C, CC, CA₃, and CA₅ as recorded for different media. The measurements in each case are for spores showing typical characters.

TABLE VII.

Dimensions of 'A' and 'B' Spores in μ for Certain Strains of Cytosporina ludibunda.

		'A' Spores.			
Strain.		Standard Medium.	Malt Medium,	Coons' Medium.	Richards' Medium.
C	Length	6-16	6-13	12-16	—
	Width	2.5-3.3	2.5-3.3	2.5-4	—
CC	Length	4-12	8-10	8-16	—
	Width	3-4.5	2-3.5	2.5-3.5	—
CA ₃	Length	6-12	8-12	8-12	—
	Width	3-4	3-5	2.3-3.5	—
CA ₅	Length	8-14	9-16	9-14	—
	Width	2.5-3.5	3-4	2.5-3.5	—
		'B' Spores.			
C	Length	24-36	30-40	20-30	20-34
CC	Length	21-32	22-50	28-50	24-34
CA ₃	Length	18-40	28-36	20-34	23-35
CA ₅	Length	22-40	28-40	24-36	20-35

N.B. Width generally varies between 1μ - 2μ .

The incomplete intergradation among 'A' and 'B' spores in standard and malt media, the complete intergradation in Coons', finally the absence of 'A' spores in the Richards' medium, indicated some influence of the nutritive conditions on the production of the two types of spores. Experimental work which has been started by varying the concentration and constituents of the media, in order to elucidate the factors concerned, has however, entered only upon an initial phase. The information available relates primarily to the mycelial and growth characteristics. It is hoped to give a more detailed account in a later paper.

V. COMPARISON OF THE SALTANT STRAINS OF *CYTOSPORINA* WITH AUTHENTIC SPECIES OF *PHOMOPSIS*.

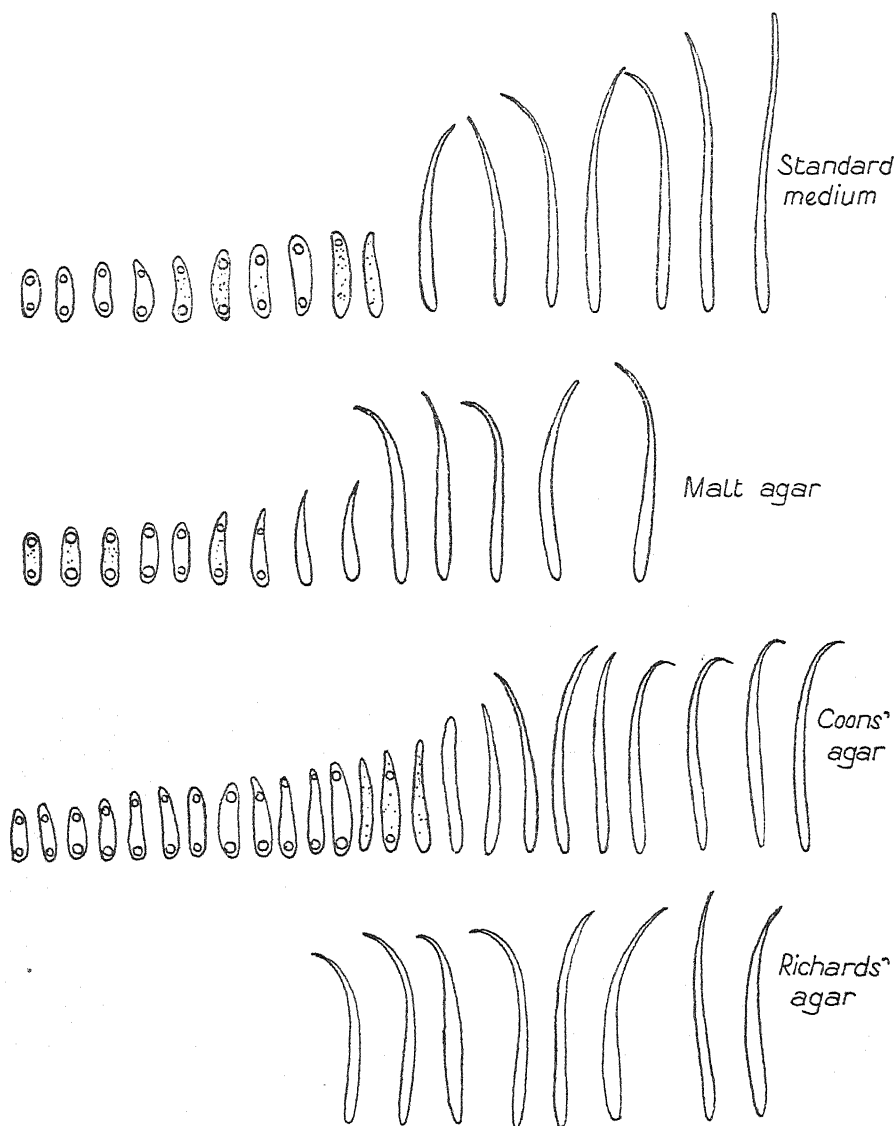
The species of *Phomopsis* available for comparative purposes were as follows:

Phomopsis coneglanensis Trav. Isolated by Archer from branch of *Aesculus hippocastanum*. (Baarn, Holland.)

Phomopsis californica. Fawcett. Isolated by Fawcett from lemon fruit and bark of lemon tree. (Baarn, Holland.)

Phomopsis citri. Fawcett. Isolated by Fawcett from Citrus. (Baarn, Holland.)

Phomopsis vexans. (Sacc. & Syd.) Harter. Isolated by Harter from *Solanum melongana*. (Baarn, Holland.)



TEXT-FIG. 6. Illustrates the spore characters of the strain CA_5 in various media.

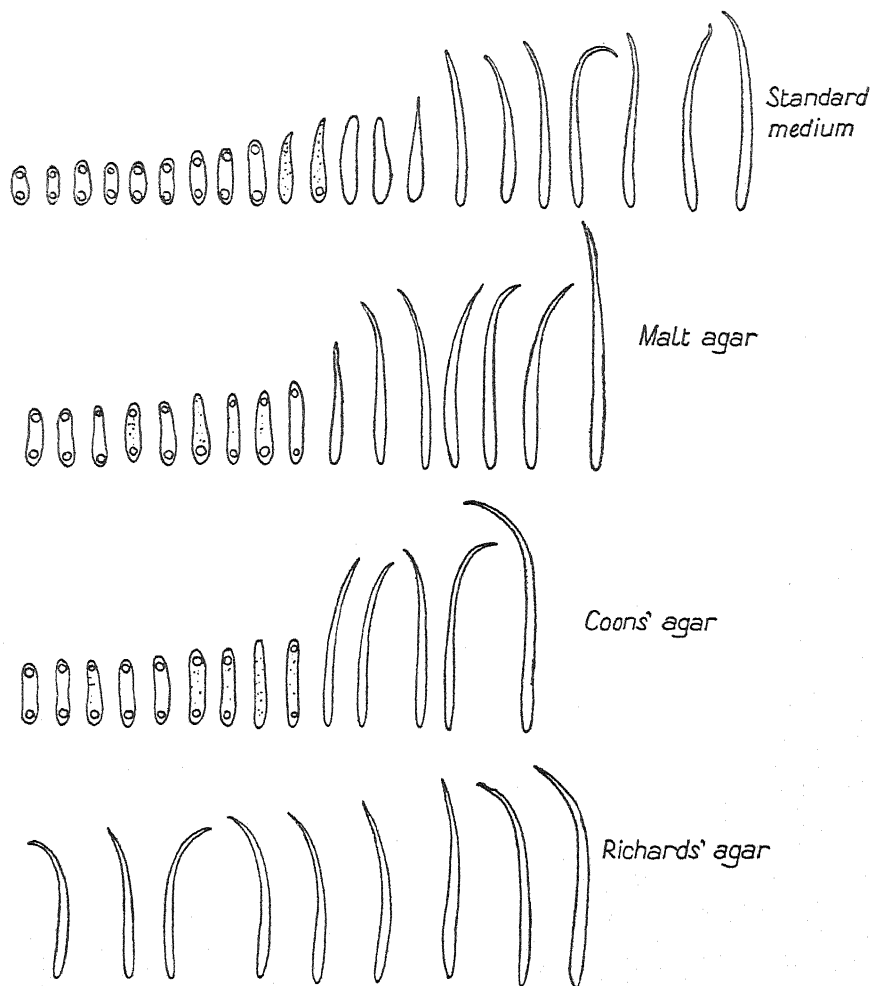
Phomopsis quercina. (Sacc.). Died. Isolated by Dulfer from branch of *Tenellis quercinus*. (Baarn, Holland.)

Phomopsis mali. Roberts. Isolated by M. N. Kidd from the apple fruit. (Received from Mr. F. T. Brooks, Cambridge.)

In addition to the above, a culture of *Cytosporina ludibunda*, referred to as CK (isolated by M. N. Kidd from the apple fruit) was sent by Mr. F. T. Brooks, Cambridge. In the first subculture of CK in standard

medium, stromata were produced which yielded both 'A' and 'B' spores.

The general characters shown by the above-mentioned strains in standard medium cultures are given below.



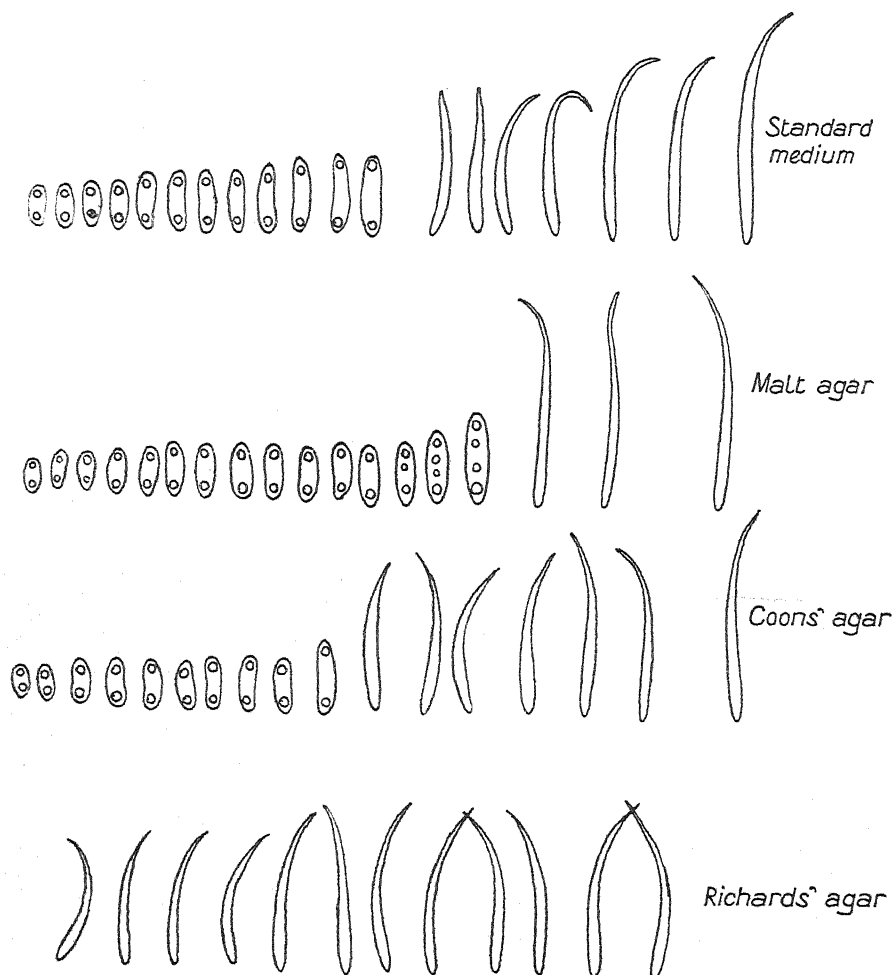
TEXT-FIG. 7. Illustrates the spore characters of the strain CC in various media.

Phomopsis coneglanensis. Aerial mycelium, forming a thick felt, white to pale yellow, darkening with age. Substratum dark brown. Zonation wide, but a narrow zone of tufted aerial mycelium occurs in the centre of the culture. Stromata numerous, dark brown, disposed in interrupted zones, and spore-heads cream containing both 'A' and 'B' spores.

Phomopsis californica. Aerial mycelium fine, silky white, turning grey with age. Substratum brown. Zonation wide, distinct. Stromata

somewhat large, dark brown, scattered, spore-heads white to cream-yellow, containing both 'A' and 'B' spores.

Phomopsis citri. Mycelium superficial, white, showing regular wide



TEXT-FIG. 8. Illustrates the spore characters of the strain CA₃ in various media.

zonation. Substratum brown. The central region of the culture pale yellow, the peripheral region white. Stromata absent.

Phomopsis vexans. Aerial mycelium thin, hirsute, brown. Substratum very brown. Zonation indistinct. Stromata absent.

Phomopsis quercina. Mycelium mostly submerged, yellowish; growth extremely retarded. Zonation absent. Substratum yellowish. Stromata absent.

Phomopsis mali. Aerial mycelium silky, white, becoming brown with

age. Substratum yellowish-brown. Zonation wide, distinct. Stromata large, numerous, dark brown, disposed in zones. Spore-heads cream, containing both 'A' and 'B' spores.

Cytosporina ludibunda (CK). Aerial mycelium copious, silky, white, procumbent with age. Substratum yellow-brown. Stromata numerous dark brown, disposed in zones. Spore-heads dull white, containing both 'A' and 'B' spores.

As far as the general features are concerned the various *Phomopsis* species, with the solitary exception of *P. quercina*, do not show any characters which separate them sharply from the *Cytosporina* derivatives. In the latter case, however, the mycelium is more or less coloured in shades of yellow, brown, grey, and black, never pure white, whereas the *Phomopsis* species form white mycelium. The distinction is merely one of degree, since in *P. coneglanensis* and *P. californica* colour develops as the cultures become older; moreover, the strain of *Cytosporina ludibunda* obtained from Cambridge does produce white mycelium. *P. quercina* differs from all the species in its slower rate of growth and absence of aerial mycelium in all the media employed.

Only *P. coneglanensis*, *P. californica*, *P. mali*, and *Cytosporina ludibunda* (Kidd) produced stromata in the standard medium, and in each case both 'A' and 'B' spores were formed. On examination of a large number of stromata it was found that in each case stromata contained both kinds of spores, but the proportion varied from strain to strain. In the case of *P. coneglanensis* only a few 'B' spores were present in the stromata. The 'A' and 'B' spores were usually present as distinct types, transitional forms being rarely observed in standard medium cultures.

The dimensions of 'A' and 'B' spores are given in Table VIII, where the limiting range in length and width and the mean dimensions are given.

TABLE VIII.

Dimensions of 'A' and 'B' Spores in Certain Species of Phomopsis.

Fungus.	'B' Spores.		'A' Spores.	
	Limiting Range in μ .	Mean Dimension in μ .	Limiting Range in μ .	Mean Length in μ .
<i>P. coneglanensis</i>	15-18	17	6-9 \times 3.5-4.0	7.5 \times 3.5
<i>P. californica</i>	25-32	27	7-12 \times 2.5-4.0	9.5 \times 3.5
<i>Cytosporina ludibunda</i> (Kidd)	24-32	30	8-11 \times 2.0-3.5	9.5 \times 3.5
<i>Phomopsis mali</i> (Kidd) . .	25-40	31	6.6-9.8 \times 3.0-3.3	7.5 \times 3.2

A comparison of Table VIII with Table IV shows :

(1) With regard to 'A' spores, the limiting range varies from 6-12 μ as opposed to the range 4-16 μ in the saltant strains. That is to say, the

In this medium the perithecial stromata were large and numerous, and developed larger necks than those found in standard medium cultures.

All the original standard medium cultures were examined for the presence of the imperfect stage, but pycnidial stromata were observed in only one culture in which perithecial stromata were also present. These stromata discharged 'A' spores. Subsequently a few cultures derived from the apparently infertile ones developed pycnidial stromata under certain experimental conditions. These stromata also discharged only 'A' spores.

Experiments were next made to discover whether the derivatives from the parent cultures showing perithecial and non-perithecial characters remained true to type. Subcultures in the standard medium were made from selected cultures belonging to each group, using mycelial inocula. In the case of the fertile group the majority of the subcultures were fertile, but a few remained infertile; in the case of the infertile group the opposite result was obtained—the majority proved infertile, a few fertile. The infertility of certain cultures was at first assumed to be due to accidental circumstances, such as drying up when the perithecia were immature, &c. This assumption, however, did not very adequately explain the variation recorded above; instead, the variation seemed related to some characteristics of the strain itself. In order to pursue the subject further work was started on new lines, using monohyphal and mono-ascospore cultures.

The monohyphal cultures were prepared from certain selected infertile cultures. Subcultures in the oatmeal agar were then made, as affording the most favourable opportunity for perithecial development. All the subcultures proved infertile. Additional experiments yielded the same result. Acting on the hypothesis that these monohyphal cultures were of unisexual nature, numerous attempts were made to cross growths derived from different parents and those derived from different parent hyphae of mono-ascospore cultures. All these attempts proved unsuccessful. A result of this kind would have been obtained if the growths were all of the same sex, but in the absence of positive evidence the question of heterothallism must be left open until further work has been carried out. It may be mentioned that Cayley (10) demonstrated that some ascospores are bisexual in certain strains of *D. pernicioso*, but reached no definite conclusion as to the occurrence of heterothallism. In the case under consideration here some of the mono-ascospore cultures produced perithecia, and were therefore homothallic, others on the contrary remained infertile.

The first standard medium cultures derived from growths of monohyphal origin showed considerable similarity, such differences as there were being related to the degree of mycelial colouring, the nature of the sterile stromata, &c. These cultures were termed DH_P. It was from one of these cultures that the 'ever-saltating' strain DH_C described in some detail in an earlier paper (20) was obtained. Later, another culture produced

well-marked sectors in the malt extract medium, the sectors differing from DH_B in mycelial characters, and in the presence of numerous pycnidia which discharged only 'A' spores. Subcultures taken from the parent and sector as a rule reproduced the parent and saltant type respectively, but occasionally inocula taken from the saltant produced infertile growths. The new strain (DH_D) failed to produce pycnidia in standard medium cultures, and was accordingly subcultured from time to time in the malt extract medium. Derivatives from these cultures after a time failed to produce pycnidia.

One definite case of sectoring in mono-ascospore culture was observed. A standard medium culture showed four sectors, the sectoring starting from the centre of the plate (Pl. XX, Fig. 14). One of these sectors was strikingly different from the remainder—the aerial mycelium forming a band round the central region of the culture, and very few stromata were present. Upon subculturing from the sector a new strain DH_M was obtained. Subcultures taken from the other three sectors resembled the parent strain DH_A . These on further subculturing sectoried, giving rise to DH_A and DH_M . A strain DH_N , differing in certain respects from both DH_A and DH_M , was obtained from another ascospore.

The chief characteristics shown by the strains derived from ascospores and hyphal tips in standard medium cultures are summarized below.

- DH_A . Obtained from a single ascospore. Aerial mycelium dense, silky, white. Substratum brown. Zonation wide, distinct. Dark brown fertile perithecial stromata and infertile stromata present.
- DH_B . Of monohyphal origin, chiefly resembles DH_A (20).
- DH_C . 'Ever-saltating' strain derived from DH_B (20).
- DH_D . Derived from a culture of DH_B and in the standard medium indistinguishable from DH_B . *In malt extract medium*, mycelium superficial, yellowish-white; substratum brown; zonation absent; pycnidia numerous, scattered, brown, discharging only 'A' spores, spores fusoid $5.5-9\mu \times 2-3\mu$, with two prominent oil-drops.
- DH_M . Obtained as a sector from DH_A . Mycelium superficial, yellowish-white. Substratum yellow. Zonation feeble or absent. Dark brown fertile perithecial stromata and infertile stromata present.
- DH_N . Obtained from another single ascospore. Mycelium superficial, a wide belt of white, loose aerial mycelium round the centre. Substratum yellowish. Zonation absent. Dark brown infertile stromata, scattered, few. Brown pycnidial stromata discharging 'A' spores only.

The variants described above have all originated from the cultures of *Diaporthe perniciosa* isolated from diseased apples by Horne in this

laboratory. All the variants belonging to this group are designated by the symbol DH. The writer had an opportunity of examining strains of *D. pernicios*a obtained from the following sources, and comparing them with the DH.

DC isolated by Cayley, received from Mr. Ashby (Kew), 1927.

DK isolated by Kidd, Cambridge, received from Mr. F. T. Brooks, 1927.

DM sent by Mr. Marsh, Bristol, 1927.

DN sent by Dr. Nattrass, Bristol, 1927.

The more important general characters shown by the strains DC, DK, DH, and DN are recorded in Table IX, observations relating to the perfect stage as developed in original cultures are given in the second column. The general characters shown by the perithecial stromata in the oatmeal medium are recorded in the third; characters relating to the imperfect stage and the mycelial characters as shown in the standard medium are given in columns four and five respectively.

TABLE IX.

*Comparison of the Strains of Diaporthe pernicios*a *obtained from Different Sources.*

Strain.	Original Culture.	Perfect Stage.	Imperfect Stage.	Mycelial Characters.
DC (Cayley).	Fairly numerous stromata (Twig culture).	Absent.	Stromata numerous. 'A' and 'B' spores equally numerous, spore-heads white to yellow.	<i>Yellowish.</i> Aerial mycelium mostly prostrate, feeble zonation. Substratum light brown.
DM (Marsh).	Pycnidial stromata.	Absent.	Stromata, black, larger than in DC. 'A' and 'B' spores, the latter few, more or less straight. Spore-heads creamy yellow.	<i>Brown.</i> Aerial mycelium silky, copious, base brown. Zonation wide. Substratum dark brown.
DH _B	Perithecial stromata.	Stromata many fertile and infertile.	Stromata, more prevalent in malt agar. 'A' spores only. Spore-heads pale yellow.	<i>White.</i> Aerial mycelium, copious, silky, erect, disposed in one or more zones. Substratum dark brown.
DK (Kidd).	Perithecial stromata.	Numerous stromata.	Stromata large (in malt agar only). 'A' spores.	<i>Yellowish-white.</i> Aerial mycelium sparse. Zonation feeble. Substratum light brown.
DN (Nattrass).	Numerous perithecial stromata.	Infertile stromata very few or absent.		<i>White.</i> Aerial mycelium white: copious, zonation feeble. Substratum white.

It is clear from the table that the strains are not identical, they differ from one another in various more or less important details. The range of variation in the pycnidial stromata is interesting. DC and DM form pycnidial stromata with 'A' and 'B' spores, but in the former 'A' and 'B' spores are almost equally numerous, and the 'B' spores are usually hook shaped, whereas in the latter 'B' spores form only a small proportion of the total spores, and they are usually straight. DH_B and DK produce pycnidial stromata, which form only 'A' spores. These have usually been obtained on malt agar. Lastly, DN formed no fertile pycnidial stromata in the medium used.

The dimensions of the pycnospores in *Diaporthe* strains, where such spores are formed, are given in Table X.

TABLE X.

Variation in Dimensions of the Pycnospores in Strains of D. perniciosia obtained from Different Sources,

Strain.	'A' Spores.		'B' Spores.	
	Variation in length in μ .	Variation in width in μ .	Variation in length in μ .	Variation in width in μ .
DC	7.0-10	2.0-3.3	25-40	1-1.5
DM	6.6-10	2.5-3.5	20-35	1-1.5
DH_B	5.5-9	2-3	—	—
DK	6-10	2.5-4	—	—

As regards the spore length, there is hardly any difference between one strain and another, and a comparison of this Table with Table IV will show, that in length these spores wholly lie within the limits shown by the spores of the saltant strains of *Cytosporina ludibunda*.

VII. DISCUSSION.

The widespread occurrence of saltation in various species of fungi has in recent years been recorded by a number of investigators. In almost all the cases mentioned, however, the changes occur in the mycelium and are manifested in the form of sectors, differing from the rest of the culture in colour, nature of the mycelium, the reproductive organs, &c. In *Cytosporina ludibunda*, on the other hand, typical sectors are of rare occurrence and when present they are usually ill-defined. Such sectors do not usually breed true but on subculturing give rise to forms which differ from the sector. Very occasionally distinct strains have been obtained from regions

of cultures which show only the parental character, for example, the strain CC₂.

While saltation in the mycelium has been followed in some detail by various investigators very little work has been done on saltation in relation to spores. Stevens and Hall (27), in 1909, while investigating the effect of environment on fungi obtained two types of colonies, namely 'a type with few pycnidia' and 'a type with many pycnidia' from the plated spores of *Ascochyta chrysanthemi*. stev. These colonies, each of which could be traced back to a single spore, subsequently proved to be distinct strains. Horne (19) described the origin of several distinct strains from individual spores of a certain species of *Fusarium*. Edgerton (14) during platings from the ascospores of some species of *Gloeosporium* found that 'Instead of one kind of colony in the plate there were two and they were very distinct'. The parent fungi, in these cases, however, were not of monosporous or monohyphal origin. Therefore, while it is quite conceivable that the appearance of colonies other than normal was due to the actual saltation in the fungus, the probability of original mixture of two fungal strains and their subsequent separation during the dilution culture could not be overlooked.

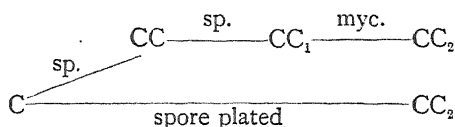
During the course of the present work on saltation in *Cytosporina ludibunda* the origin of saltants direct from the spores has been followed in greater detail. The method consisted in plating the pycnospores, which were always taken from individual spore-heads of cultures originally derived from a single hypha, and noting the different types of colonies obtained. By this way usually one, two, and occasionally as many as four variants have been obtained. In some cases the variants have proved to be distinct strains, in others, the variations observed in plates were not of a permanent nature.

This evident difference among the spores showed that the changes leading to the variations in pycnospores must have occurred at a certain stage during the development of the culture. It is quite obvious from what is known about saltation in mycelium, that such a change might occur at any time between the earliest growth stage and the formation of the mature stromata. Indeed, it is quite conceivable that in certain cases stromata may be of composite character, that is to say, compounded of the mycelia of two or more saltant strains. Very little direct evidence on this question has been obtained, but in one case the evidence is in favour of saltation during the development of spores, viz. the strain CA₃, where the colonies obtained from plating of spores, show the occurrence of a saltant strain CA₄, whereas no saltation is evident in cultures arising from the mycelium.

Usually with the saltating strains of *Cytosporina* the saltants may be obtained both from the mycelium and the pycnospores. The strains obtained by the two methods show a certain correspondence, but generally

those obtained from pycnospores vary within wider limits than do those derived direct from the mycelium.

Stevens (26) and Mahendra (24) found instances of reversion in some saltant strains of *Helminthosporium* and *Alternaria* respectively. Brown (5) working with *Fusarium* states that 'No case has been seen where, say a strain I saltated to a strain II and the latter subsequently saltated back to I'. A definite case of reversion has been encountered in *Cytosporina ludibunda*. The strain CA_5 produced as a result of sectoring the saltant CA_6 . On subculturing CA_5 from different regions of the mycelium it was found that a few of the subcultures bred true to type, but the majority have reverted back to CA_5 . In this case reversion in CA_6 does not take place uniformly throughout the mycelium, nor does it bear any relation to the age of the hyphae nor to any other outward characteristic.



TEXT-FIG. 9. Diagrammatic representation of saltation in 'jump' as shown by the production of the strain CC_2 direct from C, and again through the intermediate saltants CC and CC_1 .

The production of a saltant on one occasion by way of intermediate stages, and on another occasion in one 'jump' as noted by Brown (5) and Mahendra (24) has also been found in *Cytosporina*. Text-fig. 9 will elucidate the point.

The production of two types of colonies, 'Greys' and 'Blacks', parent and saltant types respectively—by the spores of the strain CA_3 and the gradual numerical increase of the 'Blacks' in successive sporal generations up to about 55 per cent., are features in some respects analogous to the 'Ever-sporting five-leaved clover' investigated by De Vries (11). Increase in the percentage of the five-leaved clover plants, however, was due to selection. In the case of CA_3 the selection must have been due to chance, since there is at present no means of ascertaining from the appearance of pycnospores the nature of the colonies that are likely to be produced.

A comparison of the saltant strains considered in relation to the order of their origin, reveals, in certain instances, a gradual simplification of the reproductive organs, ending generally in the total loss of fruiting character. For example, the strain CC which is characterized by the presence of large stomata gives rise to a saltant strain CC_1 which produces numerous small pycnidia; the strain CC_1 in its turn gives rise to the saltant CC_2 which does not produce any fruit bodies at all. Simplification of similar nature is also observed along another line of descent ($CA \rightarrow CA_5 \rightarrow CA_6$). But in this case no infertile saltant has been produced. It must be noted, however,

that all infertile strains do not reach that stage by gradual reduction, but that a stromatic strain may suddenly throw off a saltant which is apparently infertile.

Associated with the simplification in the reproductive organs, there is the reduction in sporulation. For example, the strain CC which produced stromata yields both 'A' and 'B' spores, while the saltant CC₁ which produced small pycnidia yields only 'B' spores. The case is exactly similar with another stromatic strain CA₅, and the pycnidial saltant CA₆ derived from it.

In general, the smaller the fruit body the fewer 'A' spores it tends to produce.

Some saltant strains (CC₁, CA₆) do not produce 'A' spores in a medium where their parents have been producing them more or less freely. When grown on other media these strains either produce only 'B' spores or remain completely infertile. From this it was naturally inferred that these strains have lost the 'A' spore-character, and can produce only 'B' spores which according to most of the investigators (4, 9) do not germinate. These strains, therefore, undoubtedly show a trend in the direction of sterility. Such sterile condition has been reached in more than one instance.

Brown and Horne (6) and Horne and Mitter (22) have found that in *Fusarium* the shape and septation of the spores can be altered by changing the composition or the concentration of the medium. In *Cytosporina*, however, the media affect the sporing in a somewhat different manner. In standard medium cultures the majority of the strains show typical 'A' and 'B' spores. In Coons' medium the strains C and CA₅ show all intermediate stages between 'A' and 'B' spores (Intergradation). Finally in Richards' medium all the *Phomopsis* type of saltants (i. e. those with 'A' and 'B' spores) produce only 'B' spores.

There is a difference of opinion as to the occurrence of intergradation between 'A' and 'B' spores. It has been shown in the course of the present work that the complete intergradation occurs only for certain strains in certain media, the partial intergradation being of more general occurrence. Although intergradation has been recorded by several investigators, its significance has not been fully realized. It finally disposes of the theory that the so-called 'B' spores are not actual spores. It is of some interest from the point of view of germination. Since according to various investigators (4, 9) the 'A' spores germinate very readily and 'B' spores do not, it is perhaps possible to discover a gradual loss of germinal power in each transitional form denoting advance towards 'B' spores.

Brown (5) found that in *Fusarium* the saltation is influenced by the medium. He states that 'the tendency of the *Fusarium* strain to saltate is a function of the cultural medium'. Mitter (25) found that a saltant strain

of *Fusarium* which had shown remarkable stability in various media for several years saltated when grown in modified standard medium with 16 and 18 per cent. glucose, and continued to do so even after its return back to original normal standard medium. In the case of *Cytosporina ludibunda*, however, the saltation does not seem to be conditioned by the medium, although it has been observed that in certain media the saltation remains masked.

The original fungus in its reproductive organs and bent filiform pycnosporoes—the 'B' spores of Diedicke—conformed to the genus *Cytosporina*. The saltant derived from *Cytosporina* yielded in addition to the filiform spores, spores of a fusoid type—the 'A' spores of Diedicke. These saltants might quite fairly be classified under *Phomopsis*. These *Phomopsis* strains differed among themselves in such characters as colour, texture and form of aerial mycelium, zonation, and also in relative proportion of 'A' and 'B' spores present in the stromata or pycnidia. Indeed, the range is so wide that two authentic species of *Phomopsis*, namely *P. coneglanensis* and *P. californica* may be easily incorporated within it. In some cases a saltant has been derived from the *Phomopsis* strains which produces the filiform ('B') spores alone, but differs from the parent *Cytosporina* in forming pycnidia instead of stromata. Such strains would in all probability find a place in the neighbourhood of *Phoma*. The most striking feature of saltation, however, is the origin of certain sterile strains, which if their origin were unknown could not be placed under any genus. That this sterility might be due to the loss of the fruiting character has been already pointed out. In a similar way might be explained the presence of various strains of *Diaporthe perniciosa*, which behave very differently in the same medium. Firstly, those that have only the perfect stage and no pycnidial stage (*D. perniciosa* isolated by Nattrass). Secondly, those that have pycnidial stage only (a) with 'A' and 'B' spores (*D. perniciosa* isolated by Marsh, Cayley), (b) with 'A' spores only (*D. perniciosa* isolated by Horne, Kidd). The first of these might have arisen by loss of the pycnidial character, a case which has often been encountered during the course of the present investigation, others by the loss of the sexual character due to saltation. Although saltation has been observed in mono-ascospore cultures of DH and some of the saltants have as yet failed to produce fertile perithecia, the writer is not in a position to definitely state the loss of sexual character in culture. Edgerton (14), however, had observed a partial loss of sexual character in a species of *Gloeosporium*. This fungus, which normally produced fertile perithecia, gave rise to a 'mutant' in which the perithecia never matured. A complete loss of sexual character has been observed by Mahendra (24) in *Neocosmospora vasinfecta*—where a saltant arising from a perithecial culture as a sector has ever since failed to produce perithecia although cultured in a number of media. Pushing this analogy further, it

may be remarked that in *Fungi imperfecti*, while some strains cannot produce a perfect stage due to the lack of suitable medium and others on account of unisexual nature, it is not improbable that there are some which have lost their sexual character by saltation, and are therefore constitutionally incapable of producing the perfect stage.

VIII. SUMMARY.

A detailed description is given of saltation in a strain of *C. ludibunda* originally isolated from diseased apples. Saltation takes place on an extensive scale—more than ten saltants have been obtained from a single monohyphal parent culture. Saltants originate from pycnosporos, from sectoring cultures, or from cultures showing no sign of sectoring. The origin of saltants from pycnosporos, as shown by the appearance of different colonies from different spores of one and the same pycnidia is a distinctive feature in this strain of *Cytosporina*, and by far the greater number of saltants has been obtained in this way. The majority of the saltants are unstable, and nearly all of those which show the constant character are infertile. The unstable saltants comprise a number of strains of 'ever-saltating' types. For example, CA₃ which produces both black and grey colonies from the same pycnidium, and CA₅ which almost always produces a sector of CA₆. In some cases saltants show reversion to parental characters.

In course of saltation the *Cytosporina* has given rise to saltants which are of *Phomopsis* type (i. e. having 'A' and 'B' spores) and some of these by further saltation have produced strains comparable to *Cytosporina* in spore character (only 'B' spores). In some cases again, a number of infertile saltants have been produced which, if their origin were unknown, could not be placed under any genus.

The variation in general morphological characters shown by the saltants in standard medium cultures covers a wide range. The majority of the saltants are of *Phomopsis* type forming both 'A' and 'B' spores. The relative proportions in which these spores are found vary from stromata to stromata as well as from strain to strain. The strains range from those with 'B' spores only to those in which such spores are rare. Some of the strains form pycnidia instead of stromata.

The character of the sporangium may be changed by altering the nutrient medium. In standard medium cultures the 'A' and 'B' spores are usually present as distinct types; in Coons' certain strains show all transitional stages between these types; in Richards' medium the same strains fail to produce 'A' spores.

The saltant strains have been compared with some authentic species of *Phomopsis*—*P. coneglanensis*, *P. californica*, &c.—and it has been found that these species do not show characters which sufficiently distinguish them from the *Cytosporina* saltants.

A description is also given of saltation in monohyphal and mono-ascospore cultures of a strain of *Diaporthe perniciosa* (DH) obtained from diseased apples. Saltants mainly differ in mycelial characters. Some of the mono-ascospore cultures produce perithecia, hence they are homothallic; others do not produce perithecia, but it is not yet established whether they are heterothallic. One monohyphal culture produced pycnidia but not perithecia.

Strains of *D. perniciosa* obtained from different sources are compared. No two strains are identical, but they differ mainly in their capacity to form the perfect and imperfect stages with regard to which the following gradation is observed. (a) Perithecia and stromata forming 'A' and 'B' spores. (b) Perithecia and stromata forming 'A' spores only. (c) Perithecia only, (d) Stromata only forming 'A' and 'B' spores. The pycnospores of *D. perniciosa* resemble those of *Cytosporina* in shape and size.

It is suggested that these *Diaporthe* strains might have originated from a common parental form by loss of sexual or asexual characters as a result of saltation.

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EXPLANATION OF PLATES XIX AND XX.

Illustrating Dr. Das Gupta's paper on Studies in the Genera *Cytosporina*, *Phomopsis*, and *Diaporthe*. II. On the Occurrence of Saltation in *Cytosporina* and *Diaporthe*.

PLATE XIX

Fig. 1. The four types of variant colonies *a*, *b*, *c*, *d*, besides the parent obtained on plating an individual spore-mass of the monohyphal parent culture C.

Fig. 2. Illustrates the characters of the saltant CA derived from variants *a* and *b* of Fig. 1.

Fig. 3. Illustrates the characters of the saltant CC derived from the variant *c* of Fig. 1.

Fig. 4. Two additional types of variant colonies *a* and *b*, besides the parent obtained on plating an individual spore-mass of CA.

Fig. 5. Illustrates the characters of CA₁ produced from the variant colony *b* of Fig. 4.

Fig. 6. Illustrates the characters of CA₂ derived from CA₁.

Fig. 7. Illustrates the character of CA₃ with a sector of CA₂.

Fig. 8. Illustrates the character of CA₄ derived from CA₁ by plating.

PLATE XX

Fig. 9. The production of two types of colonies 'Blacks' and 'Greys' from the same pycnidium of CA₃ as a result of plating.

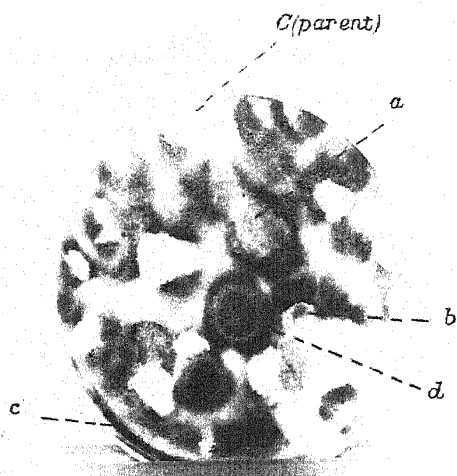
Fig. 10. The two additional types of variants *a* and *b*, besides the parent obtained on plating an individual spore-mass of CC.

Fig. 11. Illustrates the characters of CC₂ derived from the variant colonies *a* and *b* of Fig. 10.

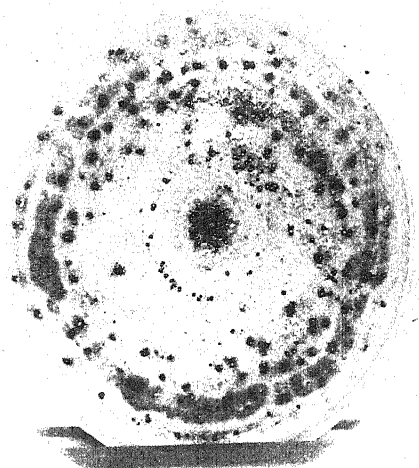
Fig. 12. Illustrates the characters of CC₁ derived from CC₁.

Fig. 13. The sectoring strain CA₅ obtained from CA ; the sector being CA₅.

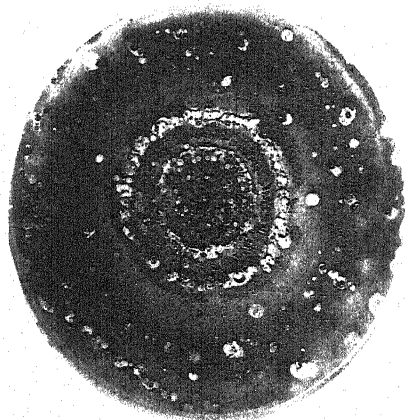
Fig. 14. The monoascospore culture of *Diaporthe pernicios*a sectoring into four; the sectors starting from the centre.



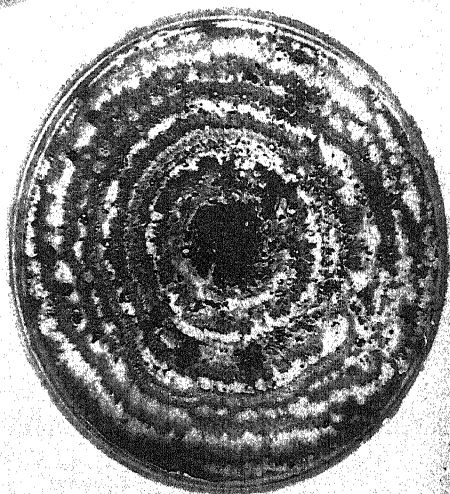
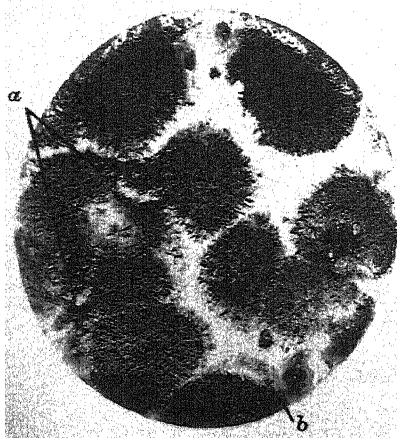
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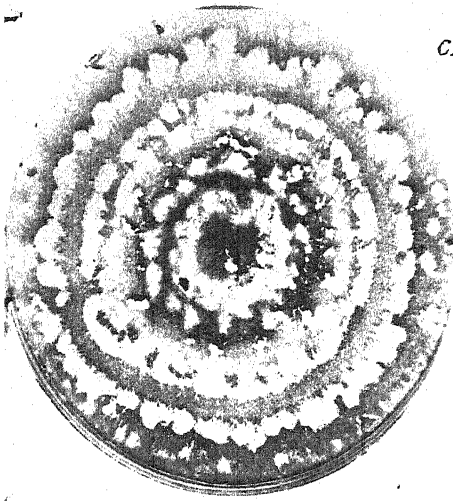


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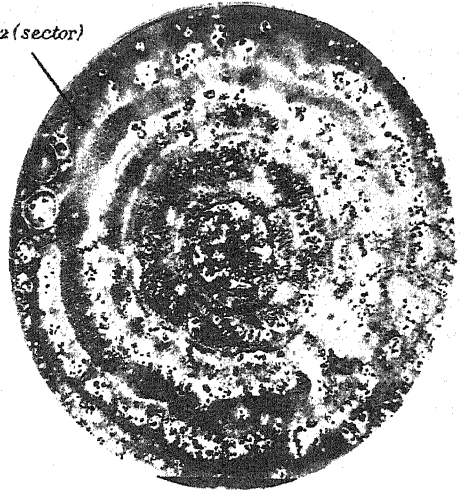
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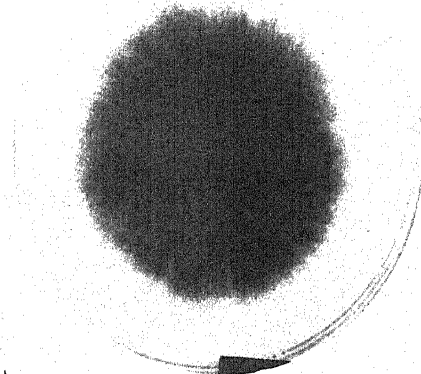


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CA₂ (sector)



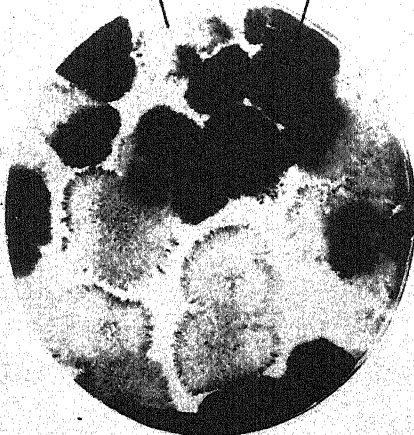
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8

CA₃ (Grays)

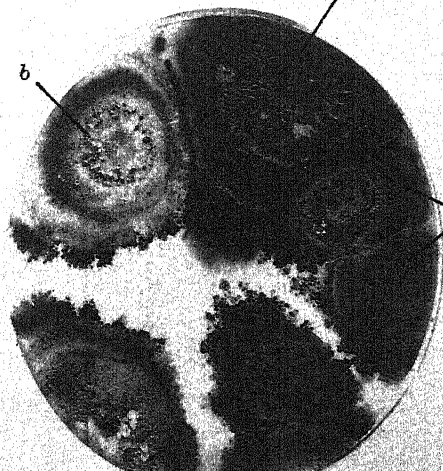
CA₄ (Blacks)

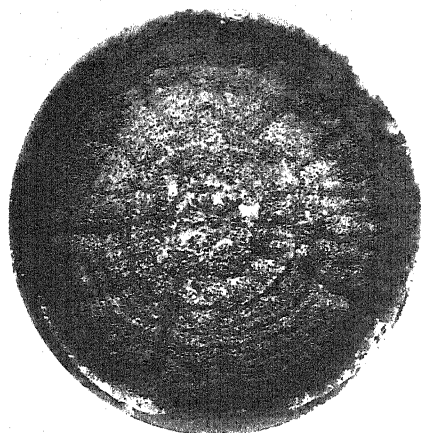


CC (parent)

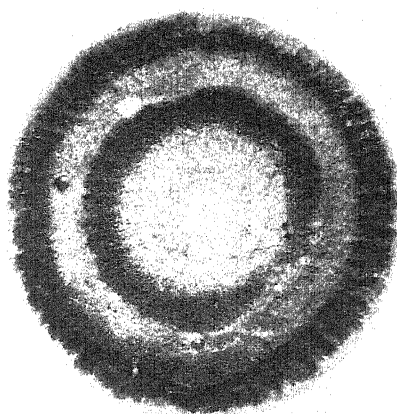
b

a

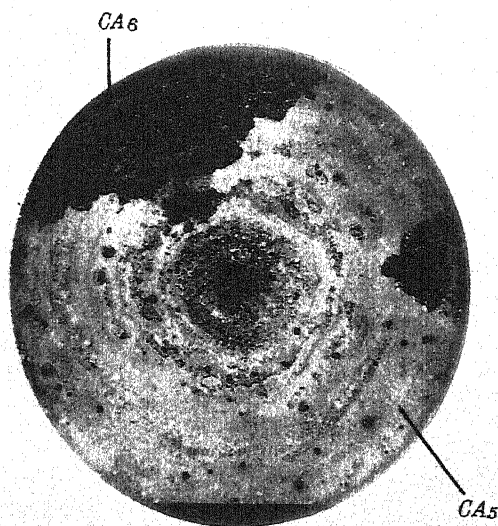




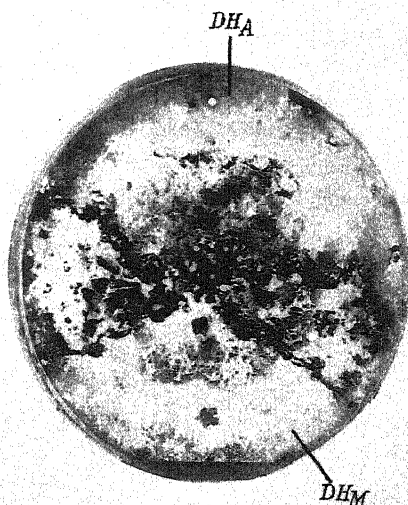
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14

The Early Development of the Root Nodule of Lucerne (*Medicago sativa*, L.).

BY

H. G. THORNTON.

(*Bacteriology Department, Rothamsted Experimental Station.*)

With Plates XXI and XXII and one Figure in the Text.

A. HISTORICAL.

MANY authors have described the histology of nodules on leguminous plants and their development. Their descriptions, however, disagree materially, while there are still important points which require elucidation. There is considerable variation in the course of nodule formation in different host legumes, which largely accounts for the disagreement. Spratt (16) studied the anatomy of nodules on some 23 genera of legumes, and divided them into four groups according to the distribution of the vascular supply, meristem, 'bacteroidal tissue', &c. Milovidov (10, 11) found important differences in the method by which bacterial infection of the host tissue is effected. He describes three types of infection. (a) In the majority of the leguminosae infection of the cells is produced by means of the characteristic 'infection threads' containing the bacteria, which intrude into the young cells produced in the meristem of the nodule. (b) In the type characteristic of *Serradella*, intercellular zoogloea plays the principal part in the infection. (c) In *Lupinus*, active division of infected cells is the principal means by which the 'bacteroidal tissue' is increased. The last type of infection is also characteristic of *Phaseolus* (8). The nodules on lucerne, here considered, belong to the first type, most, if not all the cells of the infected region receiving their bacteria by separate intrusions of the infection thread.

The general course of infection and of nodule formation in this type is well known, perhaps the best description of it being that given by Pierce (13) in the case of Burr clover. There are still important details, however, which are not understood. The bacteria normally enter the plant by penetrating the root-hairs near their distal extremity, although there is evidence that they may, more rarely, enter the other epidermal cells (3).

The plant secretes from the roots a substance which assists this infection (17), but the small percentage of root-hairs infected even in the presence of large numbers of bacteria indicates that the plant tissues can resist excessive infection (18). It is not known how the bacteria penetrate the wall of the root-hair. No cellulose-splitting enzyme can be detected in cultures of the organism, but infected root-hairs usually show a characteristic curling of the tip, which according to Hiltner (7) can be induced by means of a bacteria-free filtrate of a culture. The point of infection is generally in this curled region.

The bacteria within the root-hair form one or more 'infection threads' which grow down the hair and penetrate the cortical cells of the root. Observers have disagreed as to the structure of this infection thread. Earlier workers thought that the threads were fungal hyphae and were enclosed in a cellulose wall. Prazmowski's observations in 1890 (14), showed that the thread contained bacterial rods, and since then there have been two opinions as to their nature. Pierce (13) and Fred (6) thought that they consisted of strands of bacterial zoogloea, while (Dawson) (5), Burill and Hanson (3), and Dangeard (4) regarded the thread as a tube possessing a definite sheath containing the bacteria. The means by which the bacteria in the infection threads penetrate the cortical cell walls is not known. They often show funnel-shaped expansions at the point of contact with the wall, but opinions differ as to the significance of these.

Penetration of the cortical cells is accompanied by their rapid division, producing a mass of young cells through which the infection threads ramify. It is not clear how this cell division is induced. Some observers state that infection is limited to cells outside the endodermis, while others claim that the pericycle cells are infected. Probably this differs according to the species of host plant.

Some of the bacteria escape from the infection thread and come to lie scattered in the cytoplasm. The means by which they escape is difficult to follow, because at this stage the bacteria are very small and closely resemble the mitochondria of the cells. After their escape, the bacteria multiply rapidly and increase in size, usually becoming irregular and branched, the so-called 'bacteroid stage'. Abundance of this bacteroid stage seems to be correlated with active nitrogen fixation within the nodule.

Observers differ as to whether penetration of the cells by the infection thread immediately arrests cell division, but as soon as the bacteria become numerous in the cytoplasm the host-cell ceases to divide, but increases in size with a corresponding hypertrophy of the nucleus. The effect of the bacteria upon the infected cells has been much discussed (McCoy (8)); it is now known that this differs according to the physiology of the host plant (2) and (19), and the age of the bacteroidal tissue (9) and (19).

The following observations on the early development of the nodule in

lucerne (*Medicago sativa, L.*) were made in the hope of elucidating some of the points of uncertainty above mentioned.

B. TECHNIQUE.

Lucerne seedlings were grown in wide test-tubes containing an agar medium made up by adding the following ingredients to 1,000 c.c. of distilled water :

K_2HPO_4	.	.	.	0.5	gram.
$MgSO_4 \cdot 7H_2O$.	.	.	0.2	gram.
NaCl	.	.	.	0.1	gram.
$Ca_3(PO_4)_2$.	.	.	2.0	gram.
$FePO_4$.	.	.	1.0	gram.
$FeCl_3$.	.	.	0.01	gram.
Agar	.	.	.	10.0	gram.

The tubes of medium were sterilized in the autoclave, and each was sown with two lucerne seeds the coats of which had been sterilized by immersion in absolute alcohol followed by 0.2 per cent. $HgCl_2$, washed off with several changes of sterile water. As soon as germination took place the tubes were inoculated with a week-old culture of the lucerne nodule organism. The strain used was one of known nitrogen fixing efficiency. As soon as the first true leaves were well developed nodules began to appear, and, from this time onward, nodules of various ages were fixed in Bouin's fixative (1). Sections 5μ thick were made, and these were stained with iron haematoxylin and orange G. Some sections were also stained with carbol fuchsin, which emphasizes the bacteria but does not bring out other structures so clearly.

C. DEVELOPMENT OF THE YOUNG NODULE.

Infection was seen to have taken place through root hairs wherever sufficiently young nodules were examined (Pl. XXI, Fig. 1). Two or more infected root-hairs sometimes contributed infection threads to a single nodule. Curling of the root-hair tip was seen in all cases. The infection thread often branches within the root-hair, several strands passing down it. The threads penetrate the cortical cells as far as the inner layers, but do not enter the endodermis. Along their course the cells become more densely protoplasmic, their nuclei swell, and active cell division commences (Pl. XXI, Fig. 2). This division extends to a distance of two or three cells from the lines of infected cells, and is therefore induced by a diffusible substance. It occurs not only in the cortex but also to a small extent in the endodermis and pericycle.

The infection threads penetrate the young cortical cells produced by

division. The growing tip and youngest portions of the thread consist of a slime-like matrix filled with short rod-shaped bacteria about $0.75 \times 0.5 \mu$ in size. The infection thread has no definite sheath at this stage, its edges being somewhat irregular (Pl. XXI, Fig. 2), and there is a tendency for it to swell into zoogloea masses. When the growing tip reaches a cell wall it often swells out to form a small mass of zoogloea applied to the wall, which is penetrated at a spot somewhere near the centre of the area of contact. When the bacteria pass into the new cell they may produce a similar zoogloea mass on the other side of the wall (Pl. XXI, Fig. 5), from which the infection thread continues to grow. It is probably the subsequent shrinking of these zoogloea masses on either side of the wall that produces funnel-shaped expansions characteristic of older portions of the thread at the points where they cross cell walls (Pl. XXI, Figs. 5, 7). The growing point of the infection thread does not always swell into a mass of zoogloea at the point of contact with the wall. It may pass through the wall without changing its diameter (Pl. XXI, Fig. 4).

The infection thread, though initially naked, soon becomes enclosed in a sheath. This sheath is at first very thin, but becomes thick as the thread ages, as can be seen if the course of a thread is followed backwards from its growing point (Pl. XXI, Figs. 5 and 6). In fixed material there is usually a clear space separating the sheath from the contained strand of bacterial zoogloea (Pl. XXI, Fig. 5). This is perhaps the result of shrinkage. Older portions of the thread are sometimes almost devoid of bacteria, suggesting that the latter are able to pass down the course of the strand. This observation was also made by Dangeard (4). The sheath is continuous with and similar in staining reactions to the cell walls of the host plant, and may thus be produced by the host as a defence mechanism against the bacteria, as suggested by Moeller (12) and by Schneider (15).

The infection thread tends to grow up to and frequently applies itself against the nucleus of the host-cell, as though attracted thereto. Division of the host-cells is not immediately arrested by the entry of the bacteria (Pl. XXI, Fig. 3), since cells in a state of division can frequently be seen to contain infection threads. Such cells contain young naked portions of the thread; cell division ceases by the time the infection thread sheath is formed.

D. RELEASE OF THE BACTERIA FROM THE INFECTION THREAD.

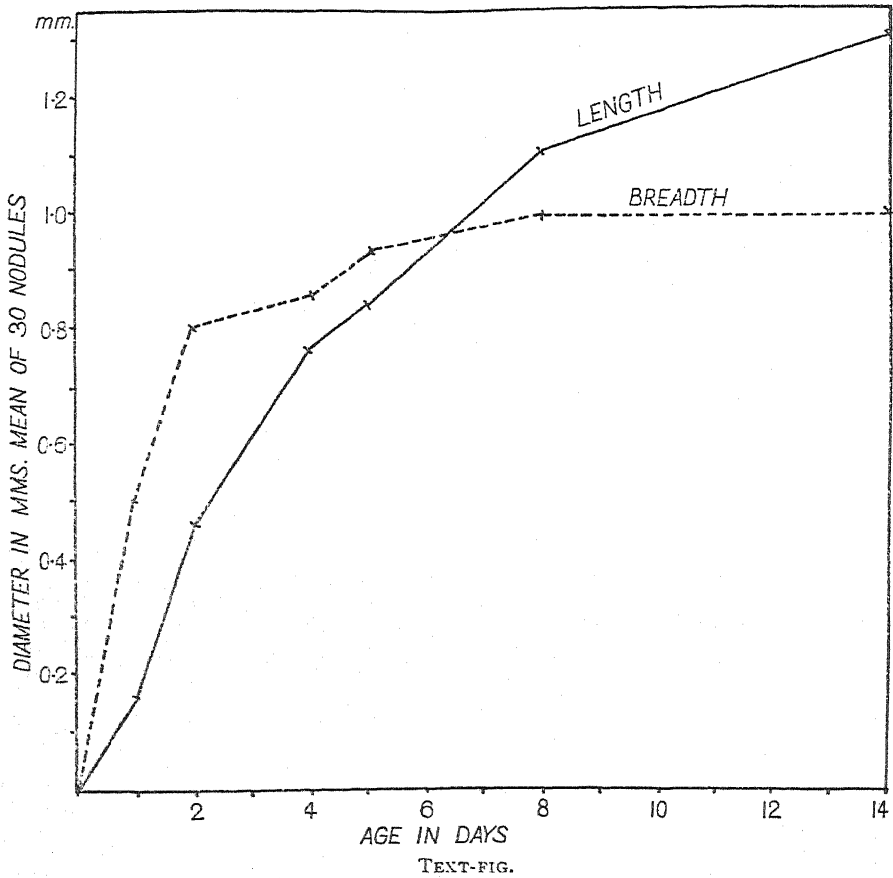
The masses of bacterial zoogloea which are produced as swellings on the young naked infection thread do not as a rule become enclosed by a sheath, but develop into flocculent masses of bacteria, from which individual coccoid rods are released and become scattered in the cytoplasm (Pl. XXII, Figs. 8, and 9). Many of the host cells thus contain scattered

bacteria at an early stage. This method of release is illustrated by Prazmowski (14) and by Dangeard (4). The bacteria are released by a different method in somewhat older cells which contain sheathed infection thread. Blister-like swellings which contain bacteria in the coccoid and short rod stage are formed on the infection thread sheath (Pl. XXII, Fig. 10). The bacteria in them do not appear to be imbedded in a stainable substance as in the tubular portions of the thread. The blisters swell out and eventually burst, releasing the bacteria into the cytoplasm (Pl. XXII, Figs. 11, 12, and 13). Milovidov (9) describes and illustrates similar structures which he calls 'cysts', in the case of *Trifolium* nodules. He states that these cysts remain unbroken until the nodule tissue becomes old, and that from them bacteria are eventually released into the intercellular spaces, where they multiply. In old lucerne nodules, portions of the infection threads persist in the cells, and it is from these that bacteria escape into the middle lamellae of the cell walls and into the intercellular spaces, eventually causing the nodule tissue to disintegrate (19). The cyst-like blisters in lucerne nodules are a means by which the bacteria are released into the young cells.

E. FORMATION OF THE BACTEROIDAL TISSUE.

The cells which have ceased to divide swell to about twice their original diameter and become vacuolated. There are at first a number of large vacuoles surrounding a central nucleus, but eventually these run together, producing a large central vacuole, which pushes the nucleus to one side (Pl. XXII, Figs. 14, 15). The latter increases in proportion to the cell, and is at first spherical. The bacteria do not appear to injure either the nucleus or the cytoplasm until the nodule tissue reaches a stage at which disintegration commences. The swelling of the cells commences in the central and proximal region of the nodule, a cap of meristem being left at the distal end. Cell division in this cap causes the nodule to increase in length, so that it becomes cylindrical. This change in proportions takes place when the nodule is about two days old (Text-fig.). An outgrowth of vascular strands from the central cylinder begins at about this time, and follows the course described in detail by Brenchley and Thornton (2) in the case of *Vicia faba*. A rapid multiplication of the bacteria in the swollen cells accompanies this outgrowth. The bacterial cells also increase in size, become banded, and eventually somewhat swollen. They do not usually develop the irregular branching forms which are characteristic of the bacteroidal tissue in many legumes. During the active period of the nodule, these somewhat enlarged banded rods constitute the bulk of the bacterial population of the nodule, the other forms of nodule bacteria being limited to the infection threads and to the region just behind the meristem cap where continuous

infection of the new cells takes place. The banded rods are thus presumably the stage which is of chief importance in fixing nitrogen.



The changes which take place as the nodule ages and which bring about its decay are described elsewhere (19).

SUMMARY AND ABSTRACT.

1. The bacteria infect the root hairs, the 'infection threads' passing into the cortex without invading the central cylinder of the root. Cell division is induced, forming a round mass of meristem cells into which the infection threads enter.

2. The infection threads are naked at their growing points and tend to swell into zoogloal masses in the cells. A sheath continuous with the wall of the host-cell is formed round the infection threads in their older portions.

3. The zoogloal masses do not become surrounded by a sheath, but

release bacteria into the host cytoplasm. At a later stage, blister-like swellings on the infection thread sheath develop, and, by their rupture, release more bacteria into the cytoplasm.

4. Dividing cells containing young infection threads occur, but division of the host-cells ceases by the time the infection thread sheath is formed. Swelling of the host-cells and multiplication of the bacteria in the cytoplasm produces the 'bacteroidal tissue' in which the bacteria become somewhat swollen banded rods. The host-cells are apparently uninjured by the bacteria save in old nodule tissue.

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EXPLANATION OF PLATES XXI AND XXII.

Illustrating Mr. H. G. Thornton's paper on the Early Development of the Root Nodule of Lucerne.

PLATE XXI.

Fig. 1. Root-hair containing infection thread (fresh material $\times 1,000$). *z.*, curled tip; *if.*, infection thread.

Fig. 2. Section of very young nodule (camera lucida drawing $\times 1,000$). *x.*, xylem of root; *m.*, meristematic cells of cortex; *if.*, infection thread; *z.*, bacterial zoogloea.

Fig. 3. Section of young nodule ($\times 500$). *c.*, cortex; *m.*, meristematic tissue; *x.*, xylem of root.

Fig. 4. Infection of a dividing cell (camera lucida drawing $\times 1,000$). *if.*, infection thread; *z.*, bacterial zoogloea; *m.*, dividing nucleus.

Fig. 5. Infection thread, *if.*, without sheath, passing through three cells. $\times 1,000$.

Fig. 6. Older infection thread with sheath, *s.* $\times 1,000$.

Fig. 7. Infection threads, *s.*, with sheaths and masses of bacterial zoogloea, *z.* $\times 1,000$.

PLATE XXII.

Fig. 8. Bacteria passing out from bacterial zoogloea, *z.* *st.*, infection thread with sheath (camera lucida drawing, $\times 1,000$).

Fig. 9. Formation of blister, *b.*, on infection thread with sheath, *st.* *bac.*, bacteria previously released into the cytoplasm (camera lucida drawing, $\times 1,000$).

Fig. 10. Swelling of blisters, *b.* *st.*, infection threads (camera lucida drawing, $\times 1,000$).

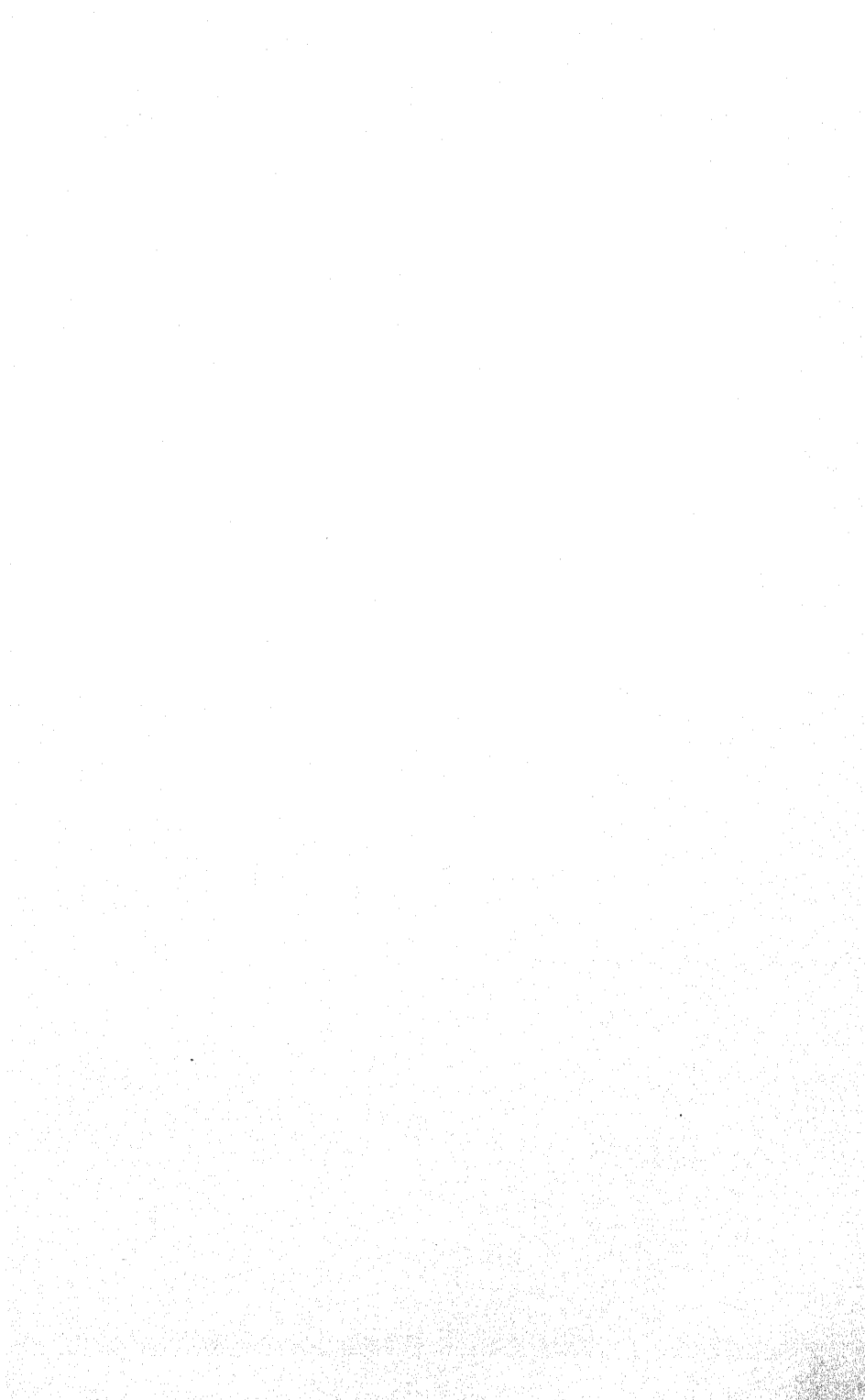
Fig. 11. Breaking of blisters, *b.*, and release of contained bacteria (camera lucida drawing, $\times 1,000$).

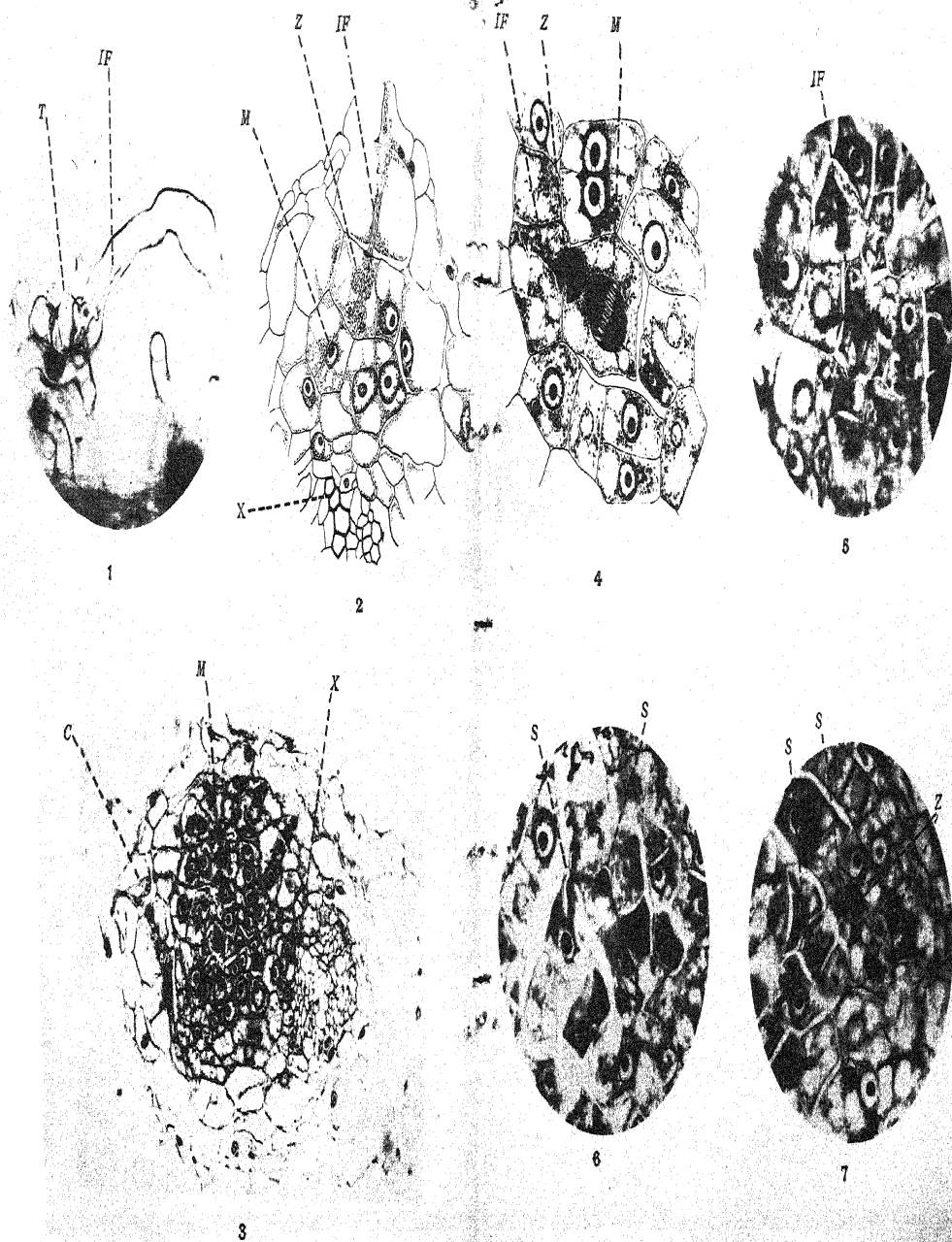
Fig. 12. Infection thread with sheath, *if.*, forming blister, *b.* $\times 1,000$.

Fig. 13. Infection thread, *if.*, with blister, *b.*, indenting the nucleus of the host-cell, *n.* $\times 1,000$.

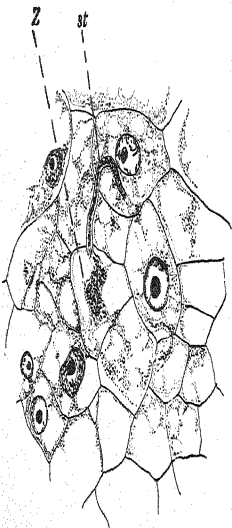
Fig. 14. Young bacteroidal tissue showing vacuoles, *v.*, surrounding host-cell nuclei, *n.* $\times 1,000$.

Fig. 15. Fully developed bacteroidal tissue showing central vacuoles, *v.*, displacing the host-cell nuclei. $\times 1,000$.

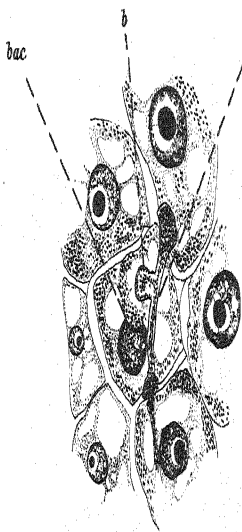




THORNTON — ROOT NODULE OF LUCERNE.



8



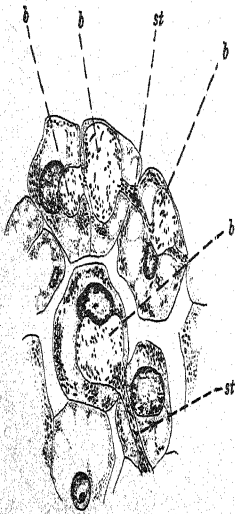
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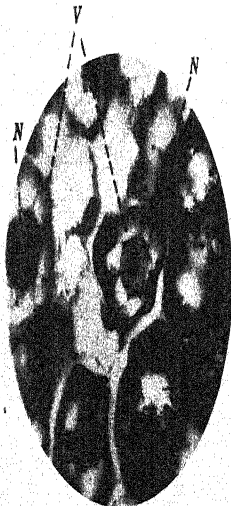
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15

On *Arceuthobium pusillum*, Peck.

I. The Endophytic System.

BY

D. THODAY,

AND

EMMA TREVOR JOHNSON.¹

(Department of Botany, University College of North Wales, Bangor.)

With Plate XXIII and nine Figures in the Text.

INTRODUCTION.

THE plants included in the genus *Arceuthobium* exhibit perhaps the highest degree of adaptation to a parasitic mode of life known in the family Loranaceae. The aerial shoot system is relatively reduced and mainly reproductive; while an endophytic vegetative system is well developed. This is especially true of *A. pusillum*, Peck (7), in which the greater part of the plant body consists of an abundantly ramifying network of strands imbedded in the tissues of the host, and the aerial shoots are smaller than in most other species of the genus.

The several species, moreover, grow exclusively on the stems of members of the Coniferae (2, p. 193). Some are restricted to a single host genus or even species: the European species, *A. oxycedri*, is only found on species of *Juniperus*, the Himalayan *A. minutissimum* only on *Pinus excelsa*. *A. pusillum* occurs principally on the one species *Picea nigra*, Link (*Picea mariana*, B.S.P.), but can spread from this to two other species of the same genus, *P. alba* and *P. rubra*, and exceptionally to *Larix americana* (16, pp. 193-5).

The species of *Viscum* and *Loranthus* are usually regarded as hemiparasites, obtaining from their hosts only water and inorganic solutes via the xylem to which they graft themselves. From his study of *A. occidentale*, on the other hand, Pierce (13) concluded that this plant must be in a position to obtain from its host diffusible organic food. The extreme reduction of the shoot system in *A. pusillum* renders a similar conclusion at

¹ This paper incorporates in part the substance of a thesis presented by the junior author in 1926 for the degree of M.Sc. in the University of Wales.

least equally probable for this species; indeed it seems unlikely that it could be to any considerable extent self-supporting. An investigation of the histological relations between the endophytic system of this parasite and the tissues of its host appeared therefore worth while, especially in view of the very interesting relations with the phloem of the host plant demonstrated by Mrs. M. G. Thoday for *Cuscuta* (15).

The material principally used in this investigation was collected by Mrs. Thoday on August 19, 1924, on an island in Lake Timagami, Northern Ontario, with such an investigation in view. We are indebted to her, not only for handing over the material to us, but also for instructing the junior author in the technique of phloem histology and giving her much help and advice during the early part of the work.

Some of the material was specially fixed in iodine for histological study, after a preliminary examination in the forest laboratory by kind permission of Professor Faull, to whom we also owe our grateful thanks for further material gathered and preserved for us the following year.

The plant is dioecious and, according to Professor Faull, male and female infections are seldom found together, or even on the same tree. The flowers open in the spring. By August the flowering male shoots have all fallen and those a year younger are mostly hidden from view. Mrs. Thoday's material was therefore exclusively female, and, in dealing with the endophytic system, we have practically confined our attention to the female plant.

Although the initial object of the present work was a histological investigation of the endophytic system, we have been led to extend it and to endeavour to frame a morphological picture of the system as a whole, its development and organization. The material available has also enabled us to fill some gaps in previous accounts of the development of the flower and fruit in the genus: this will be dealt with in a subsequent paper.

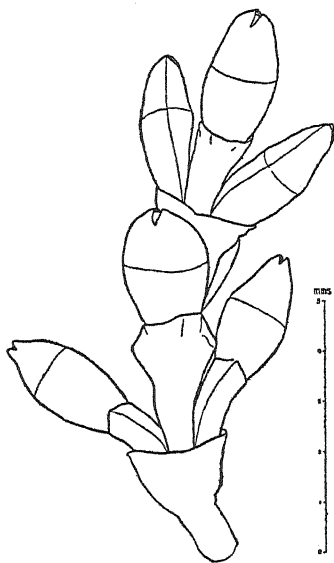
EXTERNAL CHARACTERS.

As far as can be seen externally *A. pusillum* is confined to a very definite region of the infected twigs of the *Picea*. In material collected in mid-August full-grown aerial shoots with ripening fruits occur on the three-year-old stem and occasionally on the upper part of the four-year-old stem; but as a general rule, parts more than three years old show only cup-shaped remains, each consisting of the lowest pair of leaves (occasionally a whorl of three) of a shoot which has already fallen.

The fruiting shoots, the largest of which are only about 10 mm. long, are of a dull green, with succulent jointed stems and small adpressed leaves in decussate pairs. They bear from one to five, rarely seven, fruits, one of which is usually terminal. The basal, first pair of leaves, generally barren, is succeeded by four other pairs. Of these, the fifth pair clasps the base of

the terminal ovule, the second, third, and fourth may subtend axillary ovules, most frequently the third. The most advanced shoot found is illustrated in Text-fig. 1, without its basal pair of leaves, which as usual remained attached to the host twig. As shown in the figure, fruits in the axils of the second pair of leaves may terminate short branches, themselves bearing one pair of leaves. In one exceptional case, where the main axis had been suppressed above the lowest pair of leaves, two axillary shoots had developed like normal main shoots.

On two-year-old parts of the host twig are found smaller, younger shoots with nearly mature flowers. On one year old and the current season's growth the parasite is not usually visible. Its presence is indicated, however, by the changed appearance of the twigs themselves (Pl. XXIII, Figs. 1, 2).¹ The tips are curled, the leaves are thicker and shorter than those of normal branches (6–8 mm. long as compared with 8–12 mm.), and the stems also are distinctly thicker in our material. The hypertrophy of the host twig, not being localized, but general, is not so obvious as it is in the case of such species as *A. occidentale*, where the infection is confined to a particular region of the host branch (13; cf. also 17, 18, 19: and 1, p. 101).



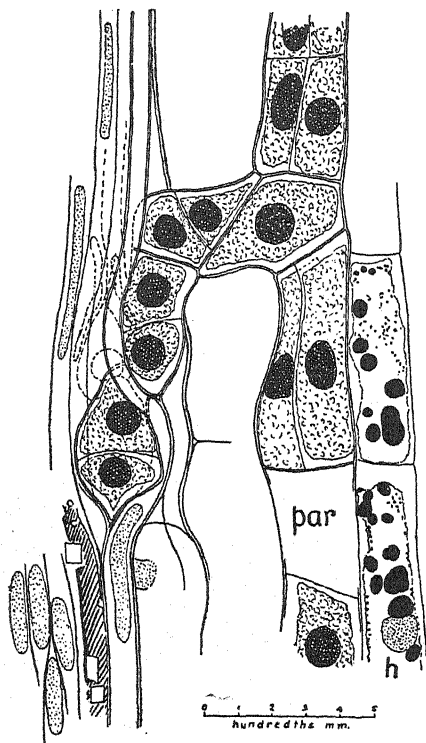
TEXT-FIG. 1. *Arceuthobium pusillum*. Fruiting shoot without its basal cup. This represents the only shoot found bearing as many as seven fruits.

THE ENDOPHYTIC SYSTEM.

Sections across fruiting parts of infected twigs show at a glance many strong strands of parasite tissue, cut in various directions, running in the inner cortex, with branches running in towards the wood and penetrating it at the medullary rays. These strands show a differentiation of tissues: outside the cells are larger, while reticulate tracheides are present in the central region. Closer examination by means of longitudinal and transverse sections reveals other strands of various diameters, some running in the inner cortex next to the phloem, others penetrating into the phloem itself or following the leaf-trace bundles on the phloem side out towards the leaf base, though not entering the leaf. They form an intricate system of ramifying and anastomosing strands. Followed upwards towards the apex of the host twig they are found strongly developed at the girdle, where

¹ We owe the first two photographs reproduced in Plate XXIII to Prof. F. E. Lloyd who very kindly lent his camera for the purpose and saw to their development.

there is often a cluster of aerial shoots. Beyond the girdle there is a sudden change to a younger continuation of the network, and similarly above each girdle the network is less elaborate and less strongly developed than below it; but slender strands can be traced in August material right into the current



TEXT-FIG. 2. *Picea nigra* (*mariana*) infected with *Arceuthobium pusillum*. Longitudinal section of twig, current season's growth, August 19, 1924; *par.*, parasite strand running longitudinally outside the host phloem, some of its cells divided longitudinally, and a branch penetrating into the phloem between the elements. Host nuclei are dotted; *h.*, host cells with resistant substance (black). The broken lines indicate underlying cells; other parts of the figure are not all at the same focus.

year's growth and have been found reaching within 2 mm. of the growing point itself.¹ Thus the endophytic system grows forward, keeping pace each season with the growth of the host twig. There is, however, a certain interval within the season between the expansion of the host buds and the invasion of the new growth by the parasite, for in material collected on July 4, 1925, the new expanding growth was apparently still free from parasite.

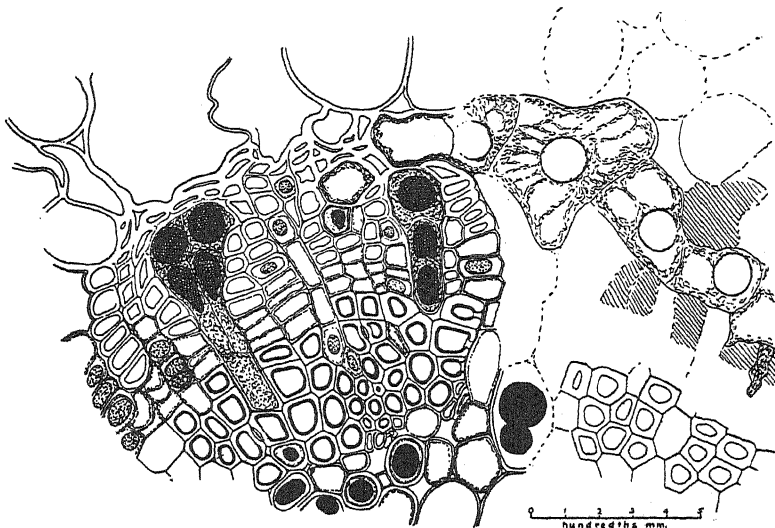
The following account of the development of the system is based on a comparison of sections from the different seasons' growth, supplemented by dissections in bulk after boiling with dilute aqueous potash. This treatment softened and loosened the bark and macerated the softer tissues within. The removal of the bark and the cortical parenchyma under a binocular dissecting microscope to disclose the parasite strands was a task requiring great care and patience, and they were often damaged in the process; but in successful dissections they stood out white by reflected light, against the darker background of translucent host tissue, owing to their

dense contents, which include a large proportion of oil.

Only in these dissections can the intricacy of the system and the thoroughness with which it penetrates the host tissues be fully grasped, or a satisfactory idea be obtained of the general arrangement of the strands and the organization of the system as a whole.

¹ In the case of *A. oxycedri*, Heinricher (6, p. 164) found at least the last 2 cm. of the host twig free from the parasite, and he remarks 'bis in die Vegetationspunkte scheint er nicht vorzudringen'.

The youngest strands, as they invade the new growth of the host twig, are simple filaments. The constituent cells, up to two or three times as long as broad, are densely filled with protoplasm and have large conspicuous nuclei. They extend at first in the inner cortex, quite near the



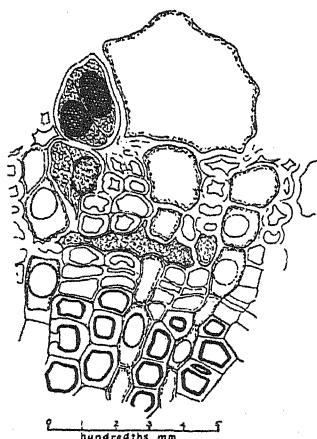
TEXT-FIG. 3. *Picea*, infected, as Text-fig. 2. Transverse section showing young 'sinks'. The fainter extension to the right, at a lower focus, shows a parasite strand, connected with one of the 'sinks' and running tangentially through medullary rays and phloem (shaded).

phloem, branching freely. Some of the principal strands run longitudinally, others follow an irregular course, often anastomosing. Other branches penetrate the phloem in various directions (Text-figs. 2, 3, and 4). By the middle of August, near the base of the current season's growth, just above the girdle, a number have penetrated to the wood to form the first young radial 'sinks'. By this time, too, most of the strands have grown in thickness. As described by Solms Laubach for *A. oxycedri* (14), the cells of the filament mostly divide very regularly by longitudinal walls. The first two divisions, at right angles to each other, result in tiers of four cells. This four-celled stage is the commonest in August of the first year, but further divisions have sometimes occurred, and immediately above the girdle the strands are generally thicker. The tiered arrangement which results from the longitudinal division of the cells is a characteristic feature, which is of the greatest assistance in distinguishing the parasite strands. It may, however, be somewhat disturbed by branching, which is often associated with the formation of oblique division walls (cf. Text-fig. 8, B). Apparently, any cell of a filament, or superficial cell of a strand, may give rise to a branch.

At the girdle, with the transition to one-year-old tissues, there is a marked increase in the amount of the parasite and, whereas above it the

strands are slender and most of the sinker-forming branches have barely reached the xylem of the host, below it there are strands five or more cells across and more numerous sinkers, mostly penetrating well into the wood (August 19).

Initiation of buds. It is here, in parts in their second season of growth,



TEXT-FIG. 4. As Text-fig. 3. Transverse section showing longitudinal strands of *Arceuthobium* in sectional view, and a tangential strand separating host sieve-tubes, &c. Compare the radial section shown in Text-fig 2

that the first signs of the formation of buds are found. In radial longitudinal sections they appear as swellings on the outer side of the biggest strands. Xylem elements appear early in the development of these buds and about the same time in the centre of associated strands. These elements are simple reticulate tracheides, of the same size and shape as the cells from which they arise.

From a study of dissections the main facts regarding the origin of buds have been made out. Growth in thickness of the strands is seldom uniform, rather variations in thickness are the rule. Here and there more conspicuous local thickening occurs, cell division for a time still being longitudinal. The more or less lenticular masses of tissue so formed may be terminal at the girdle (Text-fig. 5, A). Elsewhere they are usually intercalary.

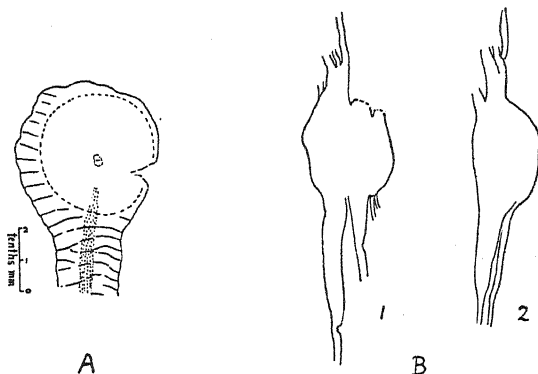
Frequently a bud is borne conjointly, as it were, on two parallel strands of more or less equal strength (cf. Text-fig. 5, B). It appears, therefore, that the mass of tissue from which a bud develops must remain plastic for a time. Whether simple or compound it broadens, in tangential view, to a rounded form, and thickens radially, taking roughly the shape of a bun with the convex side outwards.

The most distinctive sign of the differentiation of a shoot apex is the disappearance of the regular tiered arrangement of the cells on the outer face of the lenticular mass. Here, over a fairly definite rounded area, the cells are smaller and the division walls are no longer regularly longitudinal, but lie in all directions (Text-fig. 5, A). On the inner side the old arrangement still persists.

About the same time, possibly earlier, there is also a differentiation in cell contents. Whereas the inner face retains the character (in potash preparations) of the ordinary cells of the young strands, the outer side becomes translucent and yellowish or brownish in tint. The change is, at any rate partly, due to the disappearance of the reserve oil, the emulsification of which gives the strands their whitish opacity. There is further to be recorded that, when left exposed to the air in glycerine after maceration in

potash, the outer part of the bud gradually blackens (as do sections of the aerial shoot left in glycerine uncovered).

In the neighbourhood of a bud the sinkers, as well as other strands connected with a bud, grow in thickness. In the middle, cells become



TEXT-FIG. 5. A. Terminal bud of *Arcuthobium* dissected from the girdle of the host twig, viewed from the outer side; the circular area of irregular meristematic division is indicated, also the tiered arrangement of cells elsewhere. (The transverse lines are the boundaries between transverse bands of cells as seen in surface view.) The dotted lines indicate the position of tracheides, central, below the new growing point.

B. Intercalary bud, probably composite in origin, as seen (1) in front view, and (2) in side view (host surface to right), showing a number of associated strands (less magnified than A).

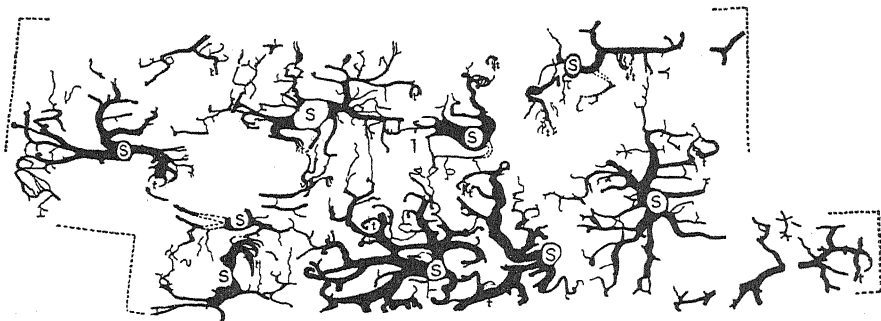
differentiated as reticulate tracheides. In the sinkers superficial cells, in contact with host tracheides, are also differentiated as tracheides, so that direct communication is provided for transport of water from the wood of the host to the aerial shoot long before it emerges into the air.

As development continues growth becomes more and more evidently related to the bud as a centre of organization (compare Pl. XXIII, Fig. 3 with Fig. 4). By the end of the fruiting season, each bud, or occasionally, if close enough together, a group of two or three buds, has radiating from its base a group of strands, simulating a root system, repeatedly branching into and connecting with finer and finer strands in the surrounding cortex, in the older phloem, and in the wood rays (Text-fig. 6, also Pl. XXIII, Fig. 4). The sharing by two shoots of a common system is not very frequent. More usually each shoot has its own system and their spatial disposition suggests a competition between them for control of the intervening ramifications.

Correlation between initiation of buds and differentiation of tracheides. The course of development as outlined above suggests that the differentiation of tracheides may not simply occur in any strand that has reached a certain thickness, but that it may wait upon the initiation of a bud. In order to obtain definite evidence on this point, we have examined dissections from second season material, using phloroglucin.

We have only found tracheides in strands connected with buds. In

the youngest stages that showed any tracheides at all they were present at the base of the buds themselves and in the stronger strands to a little distance from the buds (Text-fig. 5, A). There were often breaks in the continuity at this early stage: in particular the tracheides were often inter-



TEXT-FIG. 6. Endophytic system in a ring of rind removed from a four-year-old twig of *Picea* from which the fruiting shoots of the parasite have fallen. (The diagram should be rolled with the dotted edges meeting.) The direction of the apex of the host twig is downwards. *s.*, shoot bases from which the stronger strands radiate; *z.*, approximate positions of host leaf-traces. The larger gaps are in part due to unsuccessful dissection. No attempt has been made to represent the finer ramifications, except here and there and incompletely.

rupted at a narrower part of a strand of uneven thickness. Small buds at a relatively early stage of growth may in August show tracheides, although similar buds in July show none.

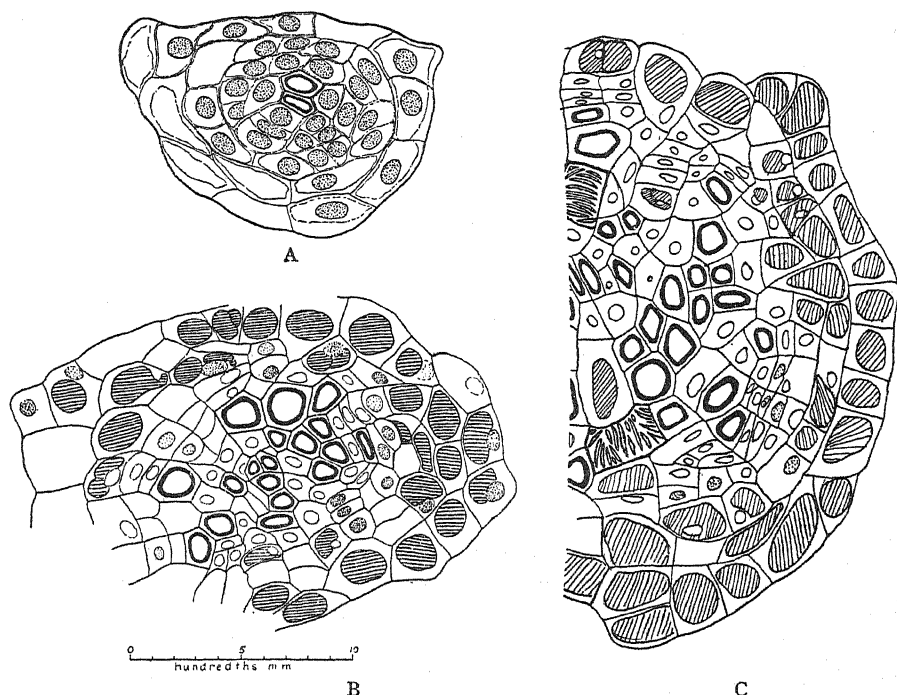
Differentiation of xylem therefore depends in part on thickness of strand, also on the seasonal cycle, but it appears to depend primarily on the development of a bud. That the initiation of differentiation should be correlated with the initiation of shoot buds is rendered the more probable by the fact that further development of the strands is so closely correlated with the growth of an aerial shoot.¹

Growth in thickness and further differentiation of strands (Text-fig. 7, A, B, C). In a transverse section of a large strand an outer zone of large cells, a few cells wide, encloses a mass of tracheides interspersed with a smaller proportion of thin-walled living cells. There is little regularity of arrangement in this central part. Sometimes there are indications of a grouping of the tracheides in blocks. Often a few tracheides are found forming a short row with one or two living cells. This is more especially the case towards the periphery where the tracheide rows are frequently continued centrifugally by two or three narrow cells.

We have not found any phloem in the strands (cf. Heinricher (6), p. 190, paragraph 13). No companion cells are cut off, nor have we been

¹ The possibility that a strand might be capable of forming xylem independently of a bud has not of course been rigidly excluded; but it remains theoretical only, for the strong strands either develop buds or are drawn into a system controlled by one.

able to demonstrate any callus, or other histological character by which sieve-tubes are recognized. The walls of the cells other than the tracheides are very thin. The staining reactions of the nuclei in the sub-peripheral



TEXT-FIG. 7. A, B, C. Strands in different stages of growth in thickness and differentiation. Stippled nuclei bluish with haematoxylin, others red with safranin. Resistant gelatinous masses of reserve product shaded.

rows, already referred to, often show a gradient (with safranin and haematoxylin) from the outermost cells, which stain bluish, to those next the tracheides, which stain red. Examination of older and younger strands shows, however, that blue-staining nuclei characterize cells still actively meristematic, and indicates that the retention of safranin is a symptom of approaching senescence. The nuclei of cells near the centre of old strands are often not only red-stained, but reduced in size.

From a comparison of strands of various ages we infer that development proceeds as follows. After the first few longitudinal divisions of a cell of the filament, the inner cells so cut off tend to become differentiated by their smaller diameter from the peripheral cells which retain the characters of the parent cell. The first tracheides are formed from cells near the centre of the strand. The short rows of tracheides indicate a tendency for successive parallel divisions to follow the differentiation of a tracheide from one

daughter cell. Very probably cell division continues to be more or less general, even at first amongst the tracheides. Later, as the central mass of differentiated tissue increases, the divisions naturally tend more and more to be parallel to the periphery. We still do not find much evidence, however, of the continuous functioning of a cambial zone. The relative lack of regularity in the arrangement of the tracheides and the absence of a sharp differentiation between the peripheral cells and those within are better explained by supposing that the peripheral cells, which obviously multiply to keep pace with the growth of the strand, also add to the central mass by a continuation of the early differentiation of narrow inner from larger peripheral cells (see especially, Text-fig. 7, B).

The vascular tissue of the aerial shoot. The anomalous structure of the endophytic strand raises at once the question whether the structure of the aerial shoot is normal, and, in particular, whether it possesses any phloem.

The groups of vascular tissue seen in transverse section bear a very close resemblance to sectors of an endophytic strand *minus* the large peripheral cells. The bulk of the group is composed of mixed xylem elements and living cells, while on its outer margin there are indications of meristematic activity here and there. The peripheral zone in which this cambial activity occurs is very meagre. Sometimes it might almost be said that tracheides were mixed up with it. Outside it again are cells, some of which are elongated and tapering, though in contents and general characters the transition seems quite gradual from these to the typical parenchyma of the cortex. None of the cells show typical phloem characters. In longitudinal section most of the elements of the vascular strand are much longer than those of the endophytic strand. This is consequential upon the fact of elongation of the aerial shoot rather than an essential difference. There are protoxylem elements, narrower tracheides with the reticulation pulled out. They show that the rather slight lignification of the thickening bands does not prevent them yielding more or less plastically.¹ The oblique common end walls of the tracheides often show a widening of the reticulation to form several round pits, a feature which is less pronounced in the endophytic strands; whether these are actual perforations has not been determined. The living cells amongst the tracheides are similar to, though more elongated than, the corresponding cells in the strands. The cambial cells are, in length as well as in other respects, very like typical dividing cells in the centre of a young strand.

To prove a negative is notoriously difficult, and before we can finally conclude that there is *no* phloem in the aerial shoot a further more systematic

¹ This is also illustrated, less conspicuously, by endophytic strands running tangentially in the cortex of the host, which have to elongate to keep pace with the growth in thickness of the stem (as is illustrated by Text-fig. 6).

study of its anatomical organization will be necessary; but our search has already been sufficiently thorough to justify us in putting forward tentatively the view that the vascular system throughout the plant is similarly organized, and that food conduction is carried on by other than typical phloem elements.

Structure of the Parasite in Relation to its Nutrition.

Before considering the nutritive relations of the parasite with its host, the question to what extent it is self-supporting requires some sort of answer. Pierce (13) found no chlorophyll in the endophytic part of *A. occidentale*. Heinricher (6) on the other hand states, for *A. oxycedri*, that the strands in the host cortex and secondary phloem and even the sinkers in the wood are bright green, though he thinks it unlikely that the chlorophyll can exercise its assimilatory function owing to the low intensity of light under the bark. Our own observations on the distribution of chlorophyll in *A. pusillum* have been confined to a dried specimen, collected and pressed in Canada, and fresh material sent over to us later, which partly dried on the way. The pressed specimen, when soaked out in warm water, showed the cortical cells of the host still bright green and the aerial shoots of the parasite olive green, but no sign of green colour in the cortical strands. In the later material the strands were yellowish, except just around the bases of aerial shoots. So far as it goes this evidence supports the view, which can be based on the conditions under which the parasite strands live, that the endophytic system is not, to any important extent at least, autotrophic. The aerial shoot, on the other hand, is provided with numerous stomata on all the exposed parts of the leaves and perianth segments; but having regard to its own growth, including especially the very considerable intercalary growth of the fruits, and to the abundance of materials stored up in practically every part of it, the flow of food, as well as of water, is more likely to be from the endophytic system to the aerial shoot than in the reverse direction.

Nutrition of the endophytic system. Any contribution that the aerial shoot might be conceived to make to the nutrition of the endophytic strands could only begin after the shoot has emerged. For the first two seasons at least the only sources possibly available to them are: (1) their own photosynthesis, (2) conduction of supplies from older parts, (3) supplies obtained directly from the surrounding host tissues.

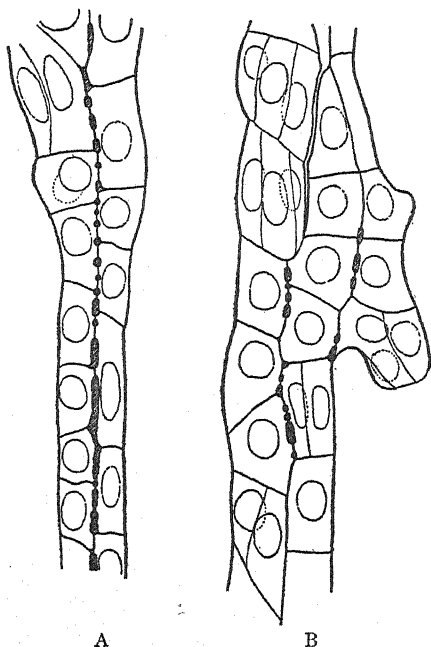
(1) Even if the strands should prove, on examination of fresh material, to be rich in chlorophyll, it is not likely that the conditions would enable them to make any important contribution to their own nutrition. The pioneer filaments in the first season run in the inner cortex. Any light that reaches them has been filtered by the fairly compact assimilatory tissue of the host stem, or, later, also by the periderm. The great reduction of

photosynthesis by a screen of chlorophyllous tissue has been sufficiently demonstrated by Willstätter and Stoll (20, p. 127).

(2) Conduction of food material along the strands is a necessary inference from the formation of the young buds, which cannot be regarded as obtaining all their food by direct absorption from the host tissues immediately surrounding them. Since the buds at first are undifferentiated masses of meristematic tissue, and the strands supplying them have not yet developed any tracheides, the ordinary cells of the strand must be capable of transporting what is required.

Consideration of the general organization of the parasite nevertheless makes it appear improbable that the younger parts depend for long or to any important extent on supplies from older parts. The way in which the aerial shoots organize their little root-like systems of strands points to them as very effective sumps towards which all supplies will tend to flow.

(3) Moreover, the details of the anatomical relations of the parasite with the host tissues point unmistakably to its dependence on the host from the beginning, for food as well as for water. In view of Mason and Maskell's



TEXT-FIG. 8. Strands of *A. pusillum*. A, in cortex; B, just outside the phloem of host (host wood towards right), showing collenchymatous thickenings and lack of correspondence of cross walls. Some cells have divided normally by longitudinal walls.

work (11, 12) it may, with some confidence, be inferred that the proximity of the young strands to the phloem, the intimate penetration of the phloem by branch strands, the close attachment of branch strands to the phloem side of incoming leaf-traces and their penetration sometimes into the phloem of the traces, all place the parasite most favourably for the absorption of assimilates coming from the leaves of the host.

The cell walls of the parasite are in general thin. The end walls of young sinkers are sometimes swollen. Rarely a local thickening of the wall, of a collenchymatous character, is observed between adjacent parasite cells: these apparently belong to a single strand, though a lack of correspondence of the cross walls suggests that two originally separate branches, happening to run alongside each other, may have fused (Text-fig. 8). Apart from such exceptional cases, however, we have not found the walls to possess the glistening collenchymatous character described by Heinricher for *A. oxy-*

cedri (6, p. 161) and by Tubeuf for the young strands in *A. pusillum* itself (16, p. 200).

The young strands exhibit features which are often associated with a haustorial function. They are plastic, insinuating themselves into the host tissues, and readily branching in almost any direction. They are rich in protoplasm, especially the branches in the phloem, and have large nuclei, features which are also characteristic of meristematic tissues. They early begin to store reserves, including oil, in considerable quantity. Thus the cells exercise the functions of penetration, absorption, and storage, synthesis of protoplasm and growth, probably also of food transport (see above, p. 404 (2)). In a sense, therefore, they are unspecialized, capable of carrying out all the functions of heterotrophic meristematic tissues. In form, too, they vary, for, though they are most commonly somewhat elongated, cells of isodiametric or irregular shapes are frequent; and while most cells are inconspicuously vacuolated or even apparently without vacuoles, some enlarge and form large vacuoles, especially outside host medullary rays and in host parenchyma, though vacuolated cells may also occur in the phloem (Text-fig. 3).

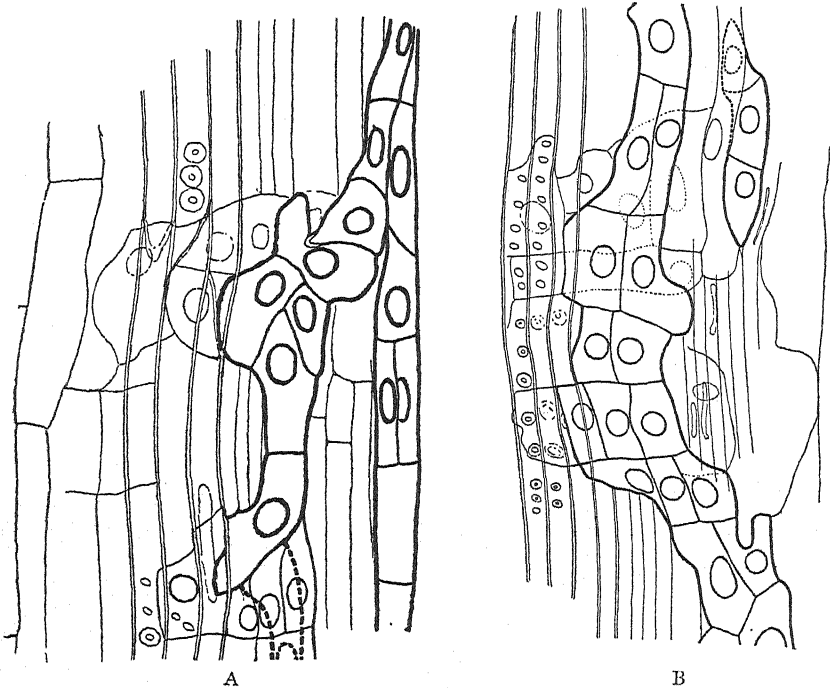
Mode of penetration and histological relations. The parasite invades the host tissues by insinuation between the cells, and we have not been able to satisfy ourselves that it ever actually enters the cell cavities. In preparations stained with ruthenium red evidence has been found of swelling and increase in pectic staining of the middle lamella in the phloem in advance of the parasite. It is probable that some general softening of the walls also occurs, and there is of necessity a certain amount of deformation of the host cells. This may account for our failure, so far, to find parasite cells applied to host sieve-plates. Nowhere does the callus stain well in the infected phloem. Yet there can be no doubt, in view of the many cases seen like those in Text-figs. 3 and 4, that the parasite does send branches between the sieve-tubes, radially as well as tangentially, and it is difficult to believe that they do not sometimes split sieve-plates. The parasite cells appear always to have walls of their own.

In the wood, when the parasite is well established, it is found in a large proportion of the medullary rays. There is no reliable evidence, however, that the developing centripetal branches, the young sinkers, ever actually penetrate beyond the cambium. Where they are found apparently just beginning to penetrate the wood it seems evident from the form of the adjacent host tracheides that these have been differentiated subsequently to penetration by the parasite between the cells of the cambial zone (cf. Text-fig. 3). We have not found the parasite in the pith; apparently it does not follow even the leaf gaps so far.

Such cases as that shown in Text-fig. 3 (confirmed in adjoining sections) indicate that the sinkers do not always penetrate by way of

medullary rays, though in old stems this appears to be the rule. The latter condition may perhaps signify that where a sinker does not follow an existing ray the adjacent cambium responds by initiating one.

We have seen one case in which the parasite, having penetrated to the wood, turned aside, and followed the margin of the xylem tangentially in



TEXT-FIG. 9. Strands of *Arceuthobium pusillum* in radial longitudinal sections of *Picea*, in July of the second year; host apex upwards; both \times about 300.

A, branch reaching the cambium at two points, obliquely, and beginning to form sinkers.

B, branch that has penetrated within the cambial zone longitudinally for a longer distance and is there widening radially to form a sinker of correspondingly greater vertical dimensions.

the cambial zone. This is evidently exceptional. It may occasionally do the same longitudinally, for in tangential sections young sinkers are sometimes found of unusual vertical extent, connecting together two, or even three, host medullary rays. More often, strands approach the cambium obliquely, and leave it again, or may enter it more than once (Text-fig. 9, A). Where they reach the cambium sinkers are formed, which in such cases tend to be ribbon-shaped and two or more cells in depth (Text-fig. 9, B).

Organization and Morphology of the Endophytic System.

The structural anomalies of the vegetative plant body we have described are such that no useful purpose can be served by ascribing it to any of the recognized morphological categories. The endophytic strands of *Arceutho-*

bium have been variously interpreted. Pierce (13) calls them modified roots, Heinricher (6) regards them as thalloid, Goebel (3) as organs *sui generis*. Engler (2, p. 162), calls them 'Rindensaugstränge' rather than 'Rindenwurzeln', though he seems to regard them as evolved from roots. The arguments that have been adduced, and the morphological parallels drawn, for the most part serve merely to emphasize selected facts without bringing us much nearer an understanding of the organization of the plant body and the mode of its evolution.

An analysis of its growth, behaviour and differentiation, with this object in view, should begin with the original infection of the host; but for *A. pusillum* we have no proper data, and for no other species are full details available. As far as can be made out from Pierce's account of *A. occidentale* an attaching disc is formed by the meristematic apex of a radicle which is undifferentiated except for a dermatogen, which takes an active part in attaching the disc to the surface of the host. From the centre of this disc grows the haustorium that penetrates through the bark into the host cortex. Anatomical details of its structure are lacking, but in the cortex, if not earlier, the superficial cells grow out as separate filaments which penetrate the host tissues in various directions and reach medullary rays. The nearest parallel to this phenomenon is the proliferation of tissues that sometimes occurs under natural or experimental conditions.

Proliferating cells cast off the yoke of a system of relations (Lang (10), pp. 708, 710), which normally would hold them to a particular course of behaviour as parts of an organ. So, too, do the initial infecting strands in *Arceuthobium occidentale*. The young strands of *A. pusillum* invading the new growth of *Picea nigra* are presumably derived from similar infecting strands. 'Proliferating meristem' aptly describes them.

Meristems are characteristically heterotrophic (or at least the more specialized meristems of higher plants). What distinguishes these strands, in the first place, from ordinary meristematic tissues is their power to penetrate the host tissues by softening the middle lamella and separating the cells. It is not essential to postulate the secretion by the parasite of solvent enzymes other than that which acts upon the walls, for their absorption of food can be accounted for otherwise. The hypertrophy of infected host tissues might well be a symptom of a disturbance of the metabolic equilibrium, whereby a larger proportion of the available supplies is kept mobilized. Such a shifting of the balance between storage and mobilization is well known as a consequence of stimulation. The concentration of diffusible nutrients would thus be increased. Stimulation has also been shown in many cases to increase the permeability of the protoplasm. If such prolonged and gentle stimulation as we have to postulate in the present instance were to maintain the permeability of the protoplasm at a higher level than the normal, diffusion would be facilitated.

No further activity would be necessary on the part of the parasite cells in the absorption of food, other than the maintenance within themselves of a low concentration of the diffusing substances by synthesis of protoplasm and reserves.

So far as our present knowledge goes, therefore, the cells of young invading strands differ from ordinary embryonic cells by the secretion of a wall-softening enzyme (probably a pectase). The early storage of reserves might be added, but this is more a question of degree.

Although this proliferating meristem has freed itself from the parent organ, there is apparent method in its proliferation. The filamentous form and acropetal advance of the primary invading strands are the first indication of this. The localization of elongation at the apex of the filaments may be regarded merely as a mechanical necessity, but the prevailing longitudinal division of their constituent cells is an indication of polarity. Even in this respect, however, they remain relatively plastic.¹ Moreover, the direction of longitudinal polarity must be reversible under the influence of a bud, when it organizes into its haustorial system strands above it as well as below.

Branching seems to be so lacking in regularity that it is simplest to regard it as occurring wherever conditions are favourable. How then are the direction of growth and the initiation of branches determined? That a gradient in essential nutrients acts as a stimulus is an obvious suggestion. Together with purely mechanical accommodation it affords a simple interpretation of many of the facts observed, though it may of course prove simpler than the truth.

We have recorded, for example, that the new host growth is not invaded immediately. During the first rapid expansion of the host, the active utilization of mobilized reserves by the host tissues keeps their concentration low. It is, probably, only when the new leaves begin to function and food accumulates, that the parasite sends branches into the new growth.

The longitudinal course mostly followed by the pioneer strands in the relatively loose inner cortex may simply mark lines of least resistance. Food materials are first absorbed from the adjacent cortical cells. This intensifies the gradient from the phloem, and branch strands turn inwards in that direction. Their course in the phloem must be influenced by directions of host-cell walls and the mode of progression of the solvent action of the parasite along the middle lamellae, but it seems too irregular for analysis.

¹ Consider, for instance the sinker in Fig. 9, B, just being initiated: ultimately the axis of the sinker and the direction of its elements will be radial.

Sinkers. The one apparent regularity is the centripetal direction of the sinkers. These radial branches penetrating the wood often differ when mature from other branches only in having peripheral tracheides, next the tracheides of the host, a feature which can be interpreted as a response to local conditions. It is their consistent direction which creates the impression that they are morphologically distinct.

It is necessary to observe here, however, that the intercalary elongation of the sinkers, in correlation with the secondary growth of the host, must necessarily be radial. The radial direction of a mature sinker is no guide to the direction followed by the branch in reaching the cambium. Unless the sinkers are formed by branches penetrating radially in response to a radial gradient to which other strands do not respond, the formation of sinkers can be regarded as a special case of the behaviour of morphologically similar strands in response to different local conditions; for, as we have already recorded, we have found no evidence that will stand scrutiny to show that sinkers literally penetrate the wood. The greater part of a sinker is the product of intercalary growth, in the host cambium.

Now the impression that the young sinker-forming branches approach the cambium radially is obtained from transverse sections such as that shown in Text-fig. 3. In this respect transverse sections may often be misleading. Branches penetrating by a radial plane, a course favoured by the structure of the host, tend to be thin tangentially and broadened vertically (cf. Text-fig. 9, B). Only in radial longitudinal sections can their direction be determined. Such sections show that strands very often approach the cambium obliquely (Text-fig. 9, A and B), and that perpendicularly radial penetration is far less frequent than might have been supposed. When it occurs, the branch seems not to be brought to a standstill merely by mechanical opposition of the wood. Such a case as that shown in the middle of Text-fig. 3, taken together with the rarity of tangential penetration in the cambial zone, suggests that the parasite loses some of its aggressiveness in a meristematic environment. That it does not reach the apex of the host can be similarly interpreted. It is almost as if in the cambium the parasite finds itself at home. There at any rate the parasite and host achieve a sort of equilibrium and grow *pari passu* with one another. Elongating now radially, its cells divide by walls parallel to the direction of their elongation, just as do the cells of the cortical filaments, and later differentiate tracheides under the influence of a bud.

The indications we have followed lead us, then, to regard the young strands of the parasite as filaments of meristematic cells, with a longitudinal polarity, which is readily modified in response to stimuli inducing branching in various directions, the master stimulus being a concentration gradient of soluble food substances.

The longitudinal polarity expresses itself not only in the prevalent cylindrical form of the cells but also in the longitudinal direction of the division walls. In these respects the cells are comparable to procambial cells.

The earliest differentiation in the thickening strand is that of inner more typically procambial cells from peripheral cells, which retain the characters of the cells of the young undifferentiated strand. The differences at first are a greater tendency to vacuolation and accumulation of reserves in the outer cells—shown in the cells of the original filaments in varying degrees—contrasted with more frequent division and smaller diameter of the inner cells and a relative absence of storage products.

This is apparently as far as differentiation proceeds until a bud arises, when a new system of relations supervenes. Starting at the base of the bud and radiating out from it along the strands connected with it, the differentiation of tracheides begins in the central core, and peripheral tracheides are also differentiated in the sinkers. Since the absence of phloem in the strands is paralleled, as far as we have discovered, in the aerial shoot, this limitation does not require any modification of the view we have suggested that the central core of the young strand is procambial in nature.

Briefly, then, the young strand before the initiation of a bud can be described as a strand of vascular meristem in a sheath of haustorial meristem with procambial features.

The mature strand comprises a core of tracheides and unspecialized xylem parenchyma, an irregular interrupted zone of cells that are still meristematic, and a sheath of storage parenchyma, the cells of which, though enlarged, vacuolated, and containing masses of the characteristic reserve substance, have probably retained for a long time a capacity for growth and division and even outgrowth into new haustorial filaments.

As to what factors are operative in determining the location of the buds we have not been able to find clear indications. The early clustering of buds in the girdle, where the host buds also form a cluster, suggests that supplies of food are specially abundant there. It seems highly probable that the best-nourished parts of the strands are the first to form buds.

Buds always arise on the outside of the strands. Of this the determining factor is probably light. There are other factors—air, water solutes—which might provide radial gradients and have, therefore, to be considered as possible stimuli; but they are likely to be less consistent in their action.

What seems clear is that the location of the buds does not follow any morphological pattern inherent in the original network of strands. The origin of a bud is rather a physiological response to internal, or a complex of external and internal, conditions. It is also a morphological event,

ushering in a new system of relations that supersedes the old. It parallels the origin of a moss plant on the protonema. It is comparable also, though not homologous, with the origin of an adventitious bud upon a root.

We do not propose in this paper to consider the vegetative system of *Arceuthobium* in relation to those of other Loranthaceae. Nor shall we enter further into its significance for causal morphology, which can be more appropriately dealt with as part of a more general discussion.

SUMMARY.

Endophytic System.

Arceuthobium pusillum lives chiefly as an endophytic parasite in the tissues of *Picea nigra*. Each season, soon after the new host growth has fully expanded, the parasite invades it and by the autumn has reached the base of the winter buds.

The invading tissues are filamentous, running more or less longitudinally at first in the inner cortex, but branching freely into the phloem and medullary rays.

They penetrate by softening the middle lamella. No reliable evidence has been found of actual entry into host-cells. Branches reach the cambium from various directions, often but not invariably beside a medullary ray, sometimes touching the cambium more than once in their irregular course. In the cambial region they grow *pari passu* with the cambium, forming radial 'sinkers' in the new wood.

The cells of the strand divide typically by longitudinal walls, recalling the behaviour of procambial cells. In the first season they are seldom more than four cells thick, except towards the base of the season's growth.

Further branching and growth in thickness occur during the second season, and buds arise as swellings on the outer side of cortical strands. Correlated apparently with the initiation of buds is the differentiation of tracheides in the centre of associated strands and sinkers; in the latter peripheral tracheides are also formed, in contact with host tracheides.

The aerial shoots emerge and the flowers are formed the following season, and in the fourth season the flowers open and the fruits are formed. Meanwhile round each aerial shoot, or occasionally round two or three close together, the strands become organized as a radiating group simulating a root system. The initiation of buds thus marks a change in the system of relations governing the organization of the endophytic system.

In cross section the strands show (1) a central core of tracheides and parenchymatous cells, (2) a peripheral zone of larger vacuolated cells which for some time retain the haustorial and meristematic features of the young strands, (3) between the two, here and there, cells still dividing, adding to

the central core. The outer zone probably goes on adding to the meristematic tissue in the actively growing strand. No phloem is present, nor has any been found in the aerial shoot.

An attempt is made to interpret the growth and development of the endophytic system on causal lines.

EXPLANATION OF PLATE XXIII.

Illustrating Professor Thoday and Miss Johnson's paper on *Arceuthobium pusillum*, Peck.

Fig. 1. Branch of *Picea nigra* infected with *Arceuthobium pusillum*.

Fig. 2. Twigs of *Picea nigra*: left, healthy; right, infected, showing curling of tips and numerous fruiting shoots of *Arceuthobium pusillum* on the three-year-old parts.

Fig. 3. Endophytic system of *Arceuthobium pusillum* before the emergence of aerial shoots. Part of a dissection, photographed by reflected light. $\times 30$. Buds were located at *b*, *b*; to the left of the centre some of the finer strands can be seen; in the strands at the right-hand top corner are indications of the tiered arrangement of the cells.

Fig. 4. Endophytic system of *Arceuthobium pusillum* in a four-year-old stem of *Picea*. Part of a dissection photographed by reflected light. $\times 5$. At *s* and *s'* are the bases of two shoots of which *s* was the stronger, with strands radiating from it all around, whereas the fewer strands connected with *s'* do not encroach on the area drawn upon by *s* (cf. Text-fig. 6); *t*, *t*, leaf-traces. At the top left-hand corner the cortical tissues and some of the phloem have been removed; the white spots are sinkers.

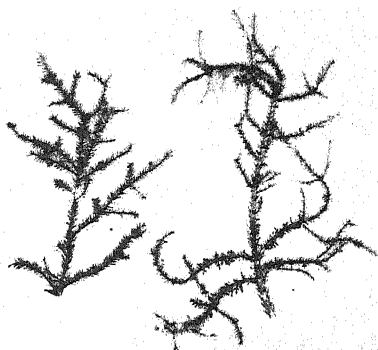
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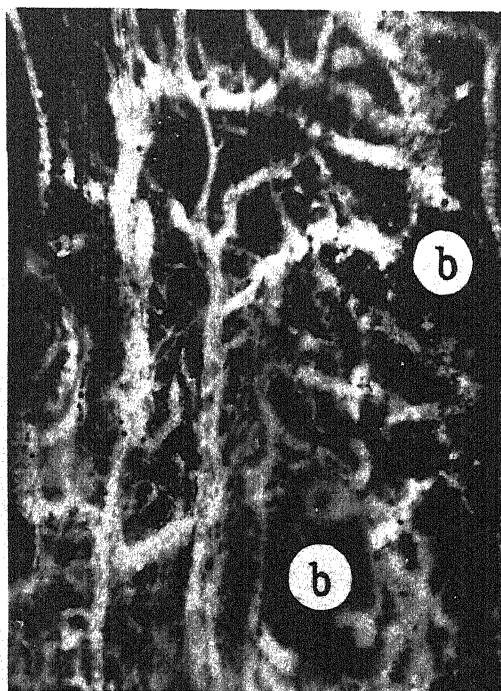
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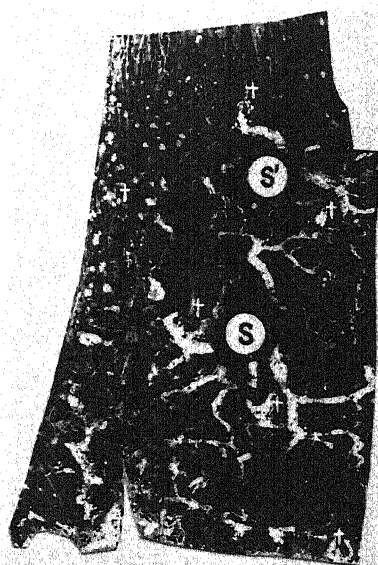
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Notes on Conifers.

V. Erect and Exserted Seeds in *Sequoia gigantea*, Torrey.

BY

W. T. SAXTON.

With four Figures in the Text.

THE erect or inverted position of the ovules in Conifers is normally constant for the genus and in some cases for the family. To a limited extent it has been used as a character of systematic importance, and it is, therefore, of interest to find that variation occurs in this respect in *Sequoia gigantea*.

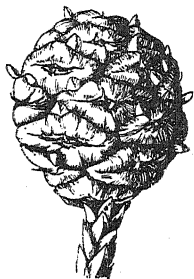


FIG. 1.

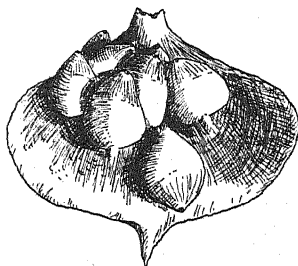


FIG. 2.

FIG. 1. Drawing (after a photograph) of a cone of *Sequoia gigantea* showing numerous erect ovules projecting between the scales. Nat. size.

FIG. 2. Drawing of a younger scale, seen from above, showing the position of the ovules (one erect, the rest inverted). $\times 8$.

The number of ovules born on a scale is usually about seven, in a somewhat poorly defined transverse double row. In most cones a few, and in some cases many, of the scales have one ovule of the distal row erect instead of inverted. As the cone grows the erect ovule grows more rapidly than the distal end of the scale, and projects outwards between the scales. Where many young seeds are found in this position a very striking appearance is presented by the cone (Fig. 1). Fig. 2 shows a younger stage in surface view of the scale from above, while Fig. 3 represents a longitudinal section of the same. Part of another longitudinal section of the same scale,

but passing through two normally oriented ovules, is shown in Fig. 4. The erect ovule is usually slightly smaller than the inverted ones.

No reference has been found to this phenomenon in the literature dealing with *Sequoia*, and when first seen in 'coning trees' of the well-known *Sequoia* Avenue near Wellington, Berkshire, in June, 1929, it was suspected of being an abnormality. In July, however, an opportunity

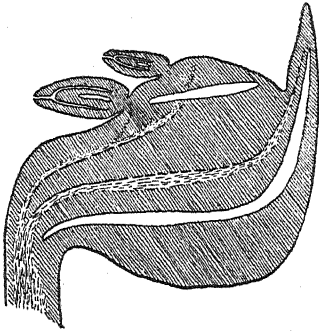


FIG. 3.

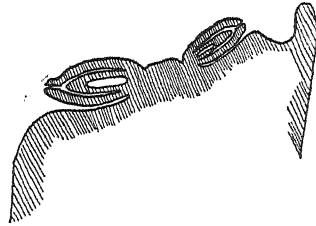


FIG. 4.

FIG. 3. Longitudinal section of a similar scale (slightly diagrammatic) showing two inverted ovules. $\times 12$.

FIG. 4. Part of a similar section (also slightly diagrammatic) showing one inverted and one erect ovule. $\times 12$.

occurred of examining a number of cones on trees in cultivation in Powerscourt Estate, Co. Wicklow. Every cone when carefully examined proved to contain erect, exserted ovules, as was the case at Wellington, so it seems clear that it must be regarded as normal for this species to have a small proportion of the ovules erect.

These facts tend to minimize the value of the position of the ovule as a character of systematic importance, at least within the Cupressaceae alliance. It seems curious that such a striking and obvious feature should not have been previously recorded, and possibly some earlier record has been overlooked. There is, however, a comparable condition which is not infrequently met with in quite a number of species of *Juniperus*, where, just as in *Sequoia*, the ovules grow much more rapidly than the scales and project between them, or in some cases even penetrate through the tissue of the scale. Full particulars, with clear figures, of this phenomenon have been given by Beck von Mannagetta und Lerchenau (1). It seems not improbable that the same exsertion of the ripe seed, as a normal condition, may account for the very curious cone of Komarov's (2) new genus *Microbiota*, which would, on this view, be very closely allied to the one-seeded asiatic species of *Juniperus* and *Sabina*, as Pilger (3) has already suggested, rather than to *Thuja* with which it does not seem to have much in common.

Thanks are due to Miss L. E. Hawker for the drawings reproduced in Figs. 1 and 2.

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Notes on Conifers.

VI. Pollination in the Pinaceae, with special reference to *Cedrus atlantica*, Manetti.

BY

W. T. SAXTON.

With four Figures in the Text.

METHODS of pollination in the Coniferae have not received a large share of attention from investigators, and less is known of pollination methods in the Pinaceae (Abietineae) than in any other family of the group. The earliest works dealing with pollination were those of Vaucher (6) and Delpino (1), and they mention only the pollination drop, which probably never occurs in the Pinaceae, though it is characteristic of all the Cupressaceae and Taxaceae. Strasburger (4) in 1871 gave an account of pollination in *Picea*, *Abies*, *Larix*, and two species of *Pinus*. In each case he found an asymmetric integument, the side adjacent to the ovuliferous scale either not developing or being shorter than the other. The whole integument is thus either like an undivided flap (*Larix*), or helmet-shaped with two or more projections at the tip, which Strasburger suspected of having a stigmatic function. Lawson (3) in 1909 described the peculiarities of the pollination of *Pseudotsuga*, but did not compare the genus with other Pinaceae or grasp its significance in relation to *Larix*, which has recently been pointed out by Doyle (2). Tison (5) in 1908 summarized the known methods of pollination in Conifers and was inclined to regard the Pinaceae as distinct from other families in this respect. The only author who refers to the pollination of *Cedrus* seems to be Wilson Smith (7) who states that he only found pollen on the scales and not in the micropyles of the ovules. He describes the micropyle as 'two-lipped', with the upper lip much longer.

In October, 1929, an opportunity occurred of examining some recently pollinated cones of *Cedrus atlantica* from a tree growing in Messrs. Waterer, Crisp and Sons' Nurseries at Bagshot, and the writer is much indebted to this firm for permission to collect the material studied. A number of young cones were carefully dissected and scores of ovules examined under the

dissecting microscope. The large, yellow, winged pollen-grains are conspicuous and unmistakable against the green integument, and in every case, as far as could be seen, they were attached to the concave side of the

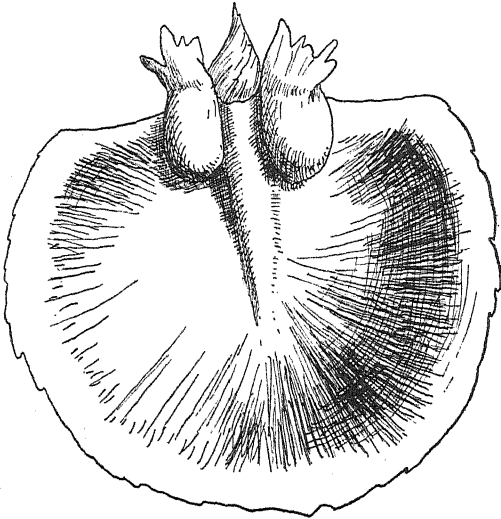


FIG. 1.

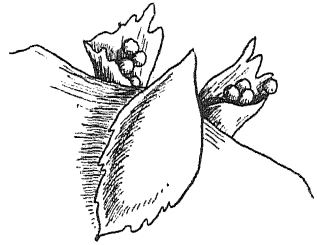


FIG. 2.

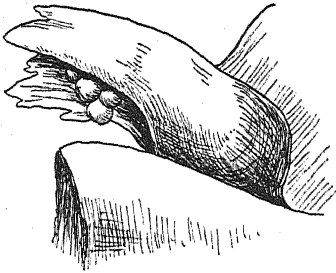


FIG. 3.

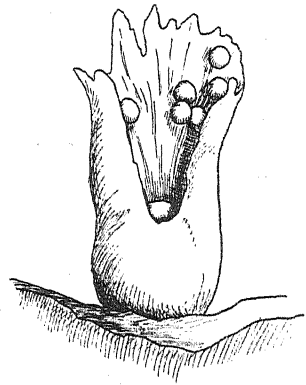


FIG. 4.

FIGS. 1-4. 1 and 2. $\times 30$. 3 and 4. $\times 60$. For explanation see text.

helmet-like integument and had not reached the nucellus. No pollen was noticed on the ovuliferous scale, but it is quite probable that a few grains might have been found there had special search been made. After removing a number of scales, masses of pollen were observed on the cone axis. Serial sections were subsequently prepared of a number of ovules, but these did not show more than two or three pollen-grains as a rule, and in some cases none. The grains found were lying free on the nucellus, exactly fitting the base of the micropyle, and showed no sign of being attached to the integument. Evidently, therefore, the processes of fixing and embedding separate the grains from the integument, and some are washed away and lost, while others get caught in the base of the micropyle. It is not unlikely that the

grains get detached from the integument in nature at a later stage, and fall into the base of the mycropyle. The appearance of these serial sections indicates how unsafe it is to rely on sections alone, as they give a wholly false impression both of the position of the pollen and of the structure of the integument.

The chief interest of the observations recorded is in showing that the integument of *Cedrus*, like those of *Larix* and *Pseudotsuga*, and in all probability of *Abies*, *Picea*, and *Pinus*, has a stigmatic function, the pollen adhering to its concave surface. As the figures indicate, quite a large number of grains get caught in this way. It is clearly not correct to describe the integument as two-lipped; it would be much more accurate to call it one-lipped. The relatively large size of the ovuliferous scale at this stage, compared to the minute bract, is also a remarkable feature, and the reverse of what is found in *Larix* and *Pseudotsuga* at a corresponding stage.

There seem good grounds for supporting Tison's (l. c.) contention that the pollination method of the Pinaceae is distinctive of the family, though observations of this feature in *Tsuga*, *Pseudolarix*, and *Keteleeria* appear to be lacking at present.

Fig. 1 is a sketch of a whole ovuliferous scale from above, showing the convex side of the integuments. Fig. 2 is a part of the same seen from below, the ends of the concave side of the integuments showing beyond the inner edge of the scale; the size and shape of the bract are also seen. Fig. 3 is a larger drawing of a similar ovule, seen from the same direction as in Fig. 2, after cutting away a part of the scale, while Fig. 4 is a side view of an ovule similarly treated to that of Fig. 3.

All the drawings were made by Miss L. E. Hawker, to whom the writer's hearty thanks are due.

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On the Metabolism of *Nematospora gossypii* and Related Fungi, with Special Reference to the Source of Nitrogen.

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With two Figures in the Text.

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[Annals of Botany, Vol. XLIV, No. CLXXIV, April, 1930.]

I. INTRODUCTION.

THE investigation to be described in the present paper arose in connexion with the use, for cultural purposes, of certain synthetic solutions. In his study of the variability of a species of *Fusarium* (*F. fructigenum*) Brown (4) prepared a synthetic medium which was approximately equivalent to potato agar, the object of doing so being to avoid the irregularity of results which the use of the more or less variable natural decoction entails. Based on a typical analysis of potato tubers, the corresponding synthetic solution should contain certain proportions of starch and reducing sugars as sources of carbon, amino-acids and proteins as sources of nitrogen, together with a number of inorganic salts. A medium of the following composition was used tentatively by Brown:

Peptone, 1.8 grm.; asparagin, 1.8 grm.; glucose, 2.0 grm.; potato starch, 40.0 grm.; potassium chloride, 0.15 grm.; magnesium sulphate, 0.75 grm.; potassium phosphate (K_3PO_4), 1.35 grm.; trace of ferric chloride; agar powder, 15 grm.; water, 1 litre.

By a series of subsidiary experiments it was shown that several of the constituents of this rather complex medium could be omitted without materially affecting the type of growth produced by the *Fusarium* concerned. The simplified medium finally adopted for standard purposes was as follows:

Glucose, 2 grm.; asparagin, 2 grm.; potassium phosphate, 1.25 grm.; magnesium sulphate, 0.75 grm.; agar, 15 grm.; water, 1 litre.

On this medium the growth of *F. fructigenum* was quite comparable, both quantitatively and qualitatively, with that on a potato decoction of suitable strength. Thus the synthetic solution contained the essential constituents of potato juice so far as the growth of *F. fructigenum* was concerned.

The simplified solution given above was found to be equally suitable for the growth of other species of *Fusarium* and of numerous other fungi. With some, however, it proved to be less suitable, and cases were met with in which it was practically useless. The fungi of the present paper are examples of the latter sort. They grow vigorously on potato extract agar, but on the simplified synthetic medium growth is much reduced, and with some species is negligible in amount. When the peptone constituent is restored to the synthetic medium, growth is vigorous and comparable to that on potato extract.

It was clear therefore that, while a fungus such as *F. fructigenum* was able to grow in a normal manner with asparagin as the sole source of

nitrogen, there were others which were unable to do so, but which apparently required a more complex nitrogenous constituent to be present in the nutrient medium. The primary object of this investigation was to examine the nature of this specialization in food requirement. While the problem is one of pure physiology, it may not be inappropriate to point out that its solution may shed some light on the problem of parasitic specialization, if the latter, as seems likely, should prove to have its basis in specialization of nutrition.

The fungi used in this work are interesting from another point of view. From their occurrence in relation to insect punctures they are described by Ashby and Nowell (2) as 'Fungi of Stigmatomycosis'. They present peculiar morphological characters, which on one hand suggest a relationship with the Phycomycetes, and on the other with some Ascomycetes such as Yeasts. The latter view is the one more commonly held (Gäumann, 7). While definite evidence on this point obviously could only be had from morphological or cytological studies, it was thought worth while to test the fermentative capacity of these organisms. A study of their behaviour to various carbohydrates was accordingly included in this investigation.

II. FUNGI USED.

The four organisms used were as follows:

1. *Spermophthora gossypii*, Ashby and Nowell.
2. *Nematospora coryli*, Peglion.
3. *N. gossypii*, Ashby and Nowell.
4. *N. gossypii*, a strain from Tanganyika Territory, E. Africa.

The first three cultures were obtained from the West Indies through the courtesy of Mr. W. Nowell. The fourth is an organism isolated by Brown from diseased cotton bolls. In all essential morphological features it is similar to the type strain of Ashby and Nowell, but differs from the latter in its more vigorous vegetative growth and in its capacity to produce a yellow colour in culture. As regards colour formation it remains constant though from time to time it has thrown both a colourless and a more coloured saltant. Both of these show the parental vigour of growth. Partly on account of its greater vegetative vigour and partly for reasons which will be given later, the strain of *N. gossypii* from E. African cotton has been chiefly used in the work on nitrogen metabolism.

III. GENERAL EXPERIMENTAL METHOD.

For comparing the suitability of various media for the growth of these fungi, the dry-weight method was used. The basal medium adopted for the great bulk of the experiments (and for all the experiments dealing with nitrogen metabolism) had the following composition:

Glucose, 2 per cent.; K_2HPO_4 , 0.5 per cent.; $MgSO_4$, 0.25 per cent.; Agar, 2 per cent.

The nitrogenous constituent, from whatever source, was supplied in a concentration equivalent to 0.3 per cent. of nitrogen.

In order to ensure a maximum degree of regularity in the different cultures, the details of the cultural routine were standardized. The Petri dishes used were $4\frac{1}{2}$ inches in diameter and the amount of medium added to each was approximately 60 c.c. The layer of medium was thus rather deep, but this had the advantage of ensuring copious growth, if conditions were otherwise suitable, and of minimizing staling effects which tend to cause irregularity. A mycelial inoculum of the fungus was placed at the centre of each plate. In the various experiments the number of plates representing each particular nutrient condition was usually about twenty, though in some cases as many as thirty-six were used.

The cultures were incubated at 30° C. for 8–10 days, the duration varying somewhat according to the different rates of radial expansion shown under different conditions. Growth was in no case allowed to proceed beyond a stage when the advancing margin of the culture touched the edge of the Petri dish, the object being to avoid any interference to growth of a purely physical nature.

At the end of the growth period, the superficial fungal material was removed. With all the fungi except *N. coryli*, this takes the form of a superficial tough skin which can be readily peeled off without removing any of the solidified medium. *N. coryli* has the manner of growth of a yeast so that in this case the product was simply scraped off. In general the various plates were treated in sets of four, the combined yields being dried on weighed filter papers at 100° C. for 3 days. The data thus represent dry weight (grm.) per four plates.

As regards the quantitative estimation of growth the fungi of this investigation have certain unusual advantages, which may be outlined at this point. The standard method of studying fungal growth, especially from the morphological point of view, is by cultures on solid media. The substitution of a liquid medium introduces certain objectionable features;¹ e.g. the growing culture tends to become submerged, in which condition

¹ A study of the differences in growth as between cultures on liquid and on solid media has been carried out in this laboratory by Miss M. P. Hall. We are indebted to her for information on this point.

it shows abnormal morphological characters, and furthermore grows very slowly. The submerged type of growth can be avoided by using a shallow layer of medium, but this in itself limits the amount of growth possible. On the other hand, with cultures on solid media, the quantitative estimation of the amount of growth is difficult inasmuch as a certain proportion of the hyphae grow into the substratum and are not accessible for weighing. The particular merit in this connexion of the four fungi used is that they form a thick skin or layer on the surface of the medium, whereas the amount of mycelium actually within the substratum is shown by microscopic examination to be very small. Presumably the fungi are highly aerobic and therefore the development of superficial mycelium prevents any considerable invasion of the underlying medium.

A control experiment was carried out, using the Tanganyika strain of *N. gossypii*, to determine the magnitude of the error involved by ignoring that portion of the mycelium which is within the agar medium. The weight of the imbedded mycelium was estimated as follows, the method being that recommended by Hall. After removal of the superficial film of mycelium the plates were flooded with dilute hydrochloric acid in the cold for 17 hours. The medium was then dissolved in a large volume of distilled water at 80° C. and the imbedded mycelium recovered by filtration through a double layer of fine muslin. The dry weight was determined in the usual way.

It is clear that the above treatment will cause certain losses of soluble cell contents and perhaps also of some cell-wall constituents. An idea of the magnitude of this loss is obtained by determining the loss of dry weight which a known quantity of superficial mycelium undergoes when subjected to exactly the same treatment. It is a legitimate assumption that the percentage losses in the two cases will be more or less similar. The true dry weight of the imbedded mycelium can thus be approximately ascertained.

The data obtained were as follows :

Dry weight of superficial mycelium (4 plates) as determined in the usual way = 0.88 gm.

Dry weight of the same after acid treatment described above = 0.62 gm.

Therefore acid treatment diminishes dry weight in ratio 1 : 1.42.

Dry weight of imbedded mycelium (4 plates) after acid treatment = 0.021 gm.

Therefore true dry weight of the latter = $0.021 \times 1.42 = 0.03$ gm.

The last figure represents 3-4 per cent. of the weight of the superficial portion and is thus negligible. Probably the actual percentage of mycelium left within the medium is less still, as it is not certain that the acid

treatment would entirely remove all traces of agar from the imbedded mycelium.

Even apart from the negligible amount of mycelium left within the medium it is clear that the error involved in the method applies in the same sense to all the measurements. The exact weights, if they could be practically determined, would all lie slightly above those ascertained. Relative values would therefore be to a large extent unaltered, and it is with these that one is chiefly concerned.

In drawing conclusions from the failure of a given substance to support growth after a certain chemical treatment, it has to be borne in mind that the failure of growth may be due, not to the lack of suitable nutrient, but to the presence of some substance which is inhibitory or even toxic. As some of the chemical treatments were of rather a drastic type, this danger is particularly prominent. In all cases where failure of growth was recorded, a strict control was set up by adding an equivalent amount of the substance tested to a standard medium which was known to give satisfactory growth. If the growth in the control was at least as good as that in the standard medium, it was argued that the failure of the preparation in question to support growth was due to the lack of nutrient quality and not to the presence in it of inhibitory substances. Illustrations of such control tests will be given later.

IV. EXPERIMENTAL RESULTS.

1. *Growth in Relation to H-ion Concentration.*

The preparation of various media for testing purposes involved in many cases severe chemical treatments, e.g. with strong acids, so that adjustment of pH values was necessary before each medium was finally ready. It was necessary therefore to determine the response of the four fungi to the H-ion concentration of the medium. The relation between amount of growth (dry weight) and H-ion concentration is set out in Text-fig. 1. The medium used was that given on p. 426 with the addition of 2 per cent. peptone.

It is clear from the graphs that all the fungi show normal features, growth extending well into the acid and alkaline sides. The optimum in all cases is about the neutral point, so that if all the media are brought to a pH of about 7 the error due to any slight fault in adjustment is brought to a minimum. The strong growth of the strain of *N. gossypii* from E. Africa is also clearly shown.

2. *Carbon Metabolism.*

A. *Availability of Various Carbohydrates.*

The basal medium for these tests was a solution with agar containing 0.5 per cent. of sugar-free peptone together with the usual mineral salts

(v. p. 426). In order to reduce as far as possible the risk of hydrolysing the carbohydrate during the process of preparing the media, the solution of carbohydrate was steamed by itself and added to the remainder after

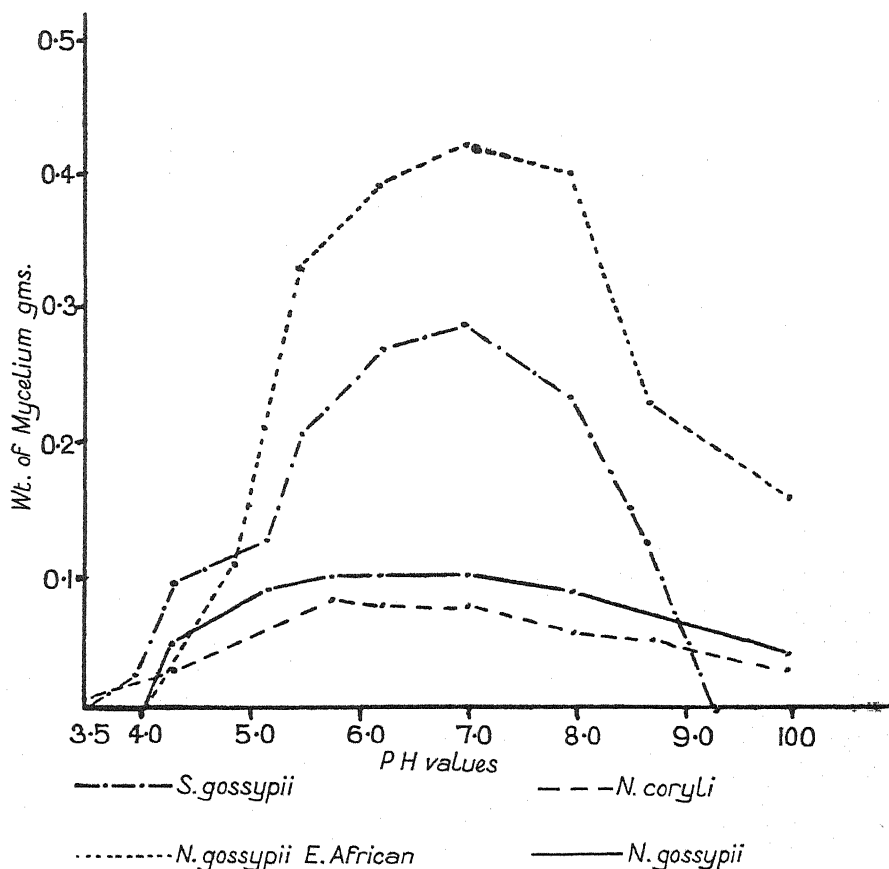


FIG. 1. Showing amount of growth on glucose-peptone-mineral salts agar at different pH values. (Temperature 21° C).

cooling. Amount of growth was determined in the usual way by weighing. The results obtained were as follows:

Hexoses. Glucose, fructose, and mannose proved to be very favourable sources of carbon, the growth on fructose being slightly greater than on the other two. Rhamnose gave only slight growth with *S. gossypii* and *N. coryli*, and quite negligible growth with both strains of *N. gossypii*. Galactose and the hexahydric alcohol, mannitol, gave negligible growth with all four fungi.

While galactose and mannitol by themselves were valueless as sources

of carbon, it was found that the addition of them to a medium containing glucose gave some increase in yield. Table I illustrates the kind of result obtained.

TABLE I.

Source of Carbon.	Yield (grm.).	
	Experiment I.	Experiment II.
1 % Glucose	0.32 ± 0.02	0.41 ± 0.016
2 % "	0.69 ± 0.064	0.83 ± 0.028
1 % " + 1 % Galactose	0.37 ± 0.024	0.60 ± 0.048
1 % " + 1 % Mannitol	0.40 ± 0.036	0.60 ± 0.064

In one experiment the increase of yield due to galactose or mannitol is not of significant amount, in the other it is just significant. In all cases the increase in yield so obtained is much less than that obtained by adding a similar quantity of glucose.

Pentoses. Neither arabinose nor xylose (2 per cent. solution) gave appreciable growth with any of the fungi.

Disaccharides. Tests were made with cane sugar, maltose and lactose. These give on hydrolysis glucose + fructose, glucose + glucose, and glucose + galactose respectively. It is not surprising, therefore, that when hydrolysed they all support good growth. When precautions were taken to avoid hydrolysis of these compounds, it was found that good growth took place on cane sugar and maltose, but practically none at all on lactose.

Starch. A sample of Lindner's soluble starch, which gave a negative test for reducing sugars when boiled with Fehling's solution, was used for this purpose. With a medium containing 2 per cent. soluble starch the growth of *N. coryli* was quite comparable to that obtained with 2 per cent. glucose, while that of the others, though very slight, was quite definite in amount. In agreement with this result, clear evidence was obtained of the excretion of diastase in cultures of *N. coryli* on a medium containing starch as sole source of carbon, whereas the evidence of such was indistinct in the case of the others.

These results are at variance with the statement of Marsh (14) that the fungi *S. gossypii*, *N. coryli*, and *N. gossypii* are unable to utilize starch. Marsh does not state whether the latter was presented in the raw or swollen form, and in any case the medium used in his test had a mixture of ammonium sulphate and nitrate as a source of nitrogen. In this work it has not been found possible to grow any of these fungi on either or both of these forms of nitrogen, so that the negative result is fully explained.

Starch which had been hydrolysed by the action of diastase gave, as was to be expected, growth comparable to that on a similar concentration of glucose. Again, it was found that, when starch was added to a medium containing glucose, the increase of growth which resulted was comparable to

that obtained by adding an equivalent amount of glucose. This was true for all four fungi. Thus while some of the latter produce diastase very slowly when starch as sole source of carbon, they produce it more freely under the more vigorous conditions of growth resulting from the presence of glucose.

B. Fermentation.

The four fungi under consideration were compared with a sample of brewer's yeast as regards their power of fermenting sugary liquids. Two solutions were used: (1), ordinary beer wort; (2), a synthetic solution containing glucose (2 per cent.), peptone (1.5 per cent.), ammonium tartrate (0.5 per cent.), K_2HPO_4 (0.5 per cent.), and $MgSO_4$ (0.25 per cent.). Cultures were set up in Durham tubes to test for the evolution of carbon dioxide, and in shallow layers of medium in flasks to give abundant aeration with a view to testing for production of alcohol. For determining the presence of the latter the boiling method of Klöcker (13) was used.

Under the conditions of the experiment, the yeast cultures gave vigorous evolution of carbon dioxide and strong production of alcohol, especially in the beer wort solution. There was no frothing with any of the other fungi and no demonstrable trace of alcohol produced, except perhaps by *Nematospora gossypii* on beer wort. The organisms under consideration, though they produce in culture a smell reminiscent of a yeast, have thus no resemblance to the latter in their metabolic activity.

3. Nitrogen Metabolism.

It has already been stated that these organisms are specialized in regard to the source of nitrogen which they can use. They can assimilate peptone but to a much less extent asparagin. It may also be added that such sources of nitrogen as potassium nitrate and ammonium salts are also of little value. In these respects it was found that the two forms of *Nematospora gossypii* showed the highest degree of specialization. *N. coryli* was not so specialized, i.e. while it grew very poorly on the various unsuitable sources of nitrogen, the relative amount of growth was greater than with the other species of *Nematospora*. *Spermophthora gossypii* was definitely the least strictly specialized of all. On account of the high vegetative vigour and of the high degree of specialization shown by it, the E. African form of *N. gossypii* was principally used in that phase of the work which concerned nitrogen metabolism.

A. Growth on peptone preparations.

The first problem was to determine whether the fungus required a fairly complex nitrogenous molecule as such, or whether it would grow on a mixture of the simple hydrolytic products. Standard methods of hydrolysis,

of recovery and estimation of the amino nitrogen, &c., were adopted as described below. These methods were used throughout the whole of the experimental work.

(a) *Hydrolysis.*

Peptone (Hopkins and Williams's preparation) was warmed gently for one hour with four times its weight of 22.5 per cent. sulphuric or hydrochloric acid,¹ after which it was heated under a reflux condenser for 24 hours. This is the standard method for hydrolysis of proteins and assures complete conversion to amino-acids. On completion of hydrolysis the liquid while still hot was neutralized in order to avoid the formation of piperazines.

In the case of hydrolysis with sulphuric acid a hot saturated solution of baryta was added after the liquid had been diluted to five times its volume. To ensure the recovery of at least 70 per cent. of the nitrogen, the precipitate was washed by boiling with distilled water. The combined filtrates and washings were made faintly alkaline, and finally the last traces of baryta were precipitated with dilute sulphuric acid. The liquid was then evaporated to a convenient volume.

When hydrochloric acid was used, the bulk of the acid was distilled off *in vacuo* until a thick pasty residue was left. The latter was made up with a suitable volume of water, after which it was neutralized either with sodium hydroxide or silver hydroxide.² The former reagent proved to be unsatisfactory as there remained in solution sufficient sodium chloride to affect deleteriously the growth of the organism. Hydrolysis with hydrochloric acid, followed by neutralization with silver hydroxide, gave a solution comparable to that obtained by the sulphuric acid. The trouble and expense involved, however, were greater. The sulphuric acid method was adopted as standard.

Figures illustrative of a typical hydrolysis are as follows:

23 grm. peptone, after treatment with sulphuric acid, precipitation with baryta and subsequent washings of the precipitate, gave about $2\frac{1}{2}$ litres of hydrolytic products. This was evaporated down to about a litre giving a solution which contained approximately 0.3 per cent. of N. The latter figure is equivalent to 20 grm. of peptone per litre.

(b) *Adjustment of pH value.*

It was found convenient to adjust the pH value of the nitrogen-containing solutions to 5.3 for two reasons: (1) if the final solution was acid

¹ Attempts were made to carry out the hydrolysis with commercial preparations of erepsin and trypsin. The difficulty with these is that hydrolysis is incomplete, and there is no practicable method of separating the hydrolysed and unhydrolysed portions.

² This reagent was freshly prepared from silver nitrate by precipitation with 40 per cent. soda, and washed free of sodium hydroxide by decantation.

rather than neutral the complete removal of basic precipitants such as baryta and silver hydroxide was simplified ; (2) the salts added later in the preparation of the culture medium neutralized this solution and gave a final pH value of 6.8. For determining pH values the colorimetric method of Clark (6) with the use of a colour chart was adopted. The method was regarded as sufficiently accurate since *N. gossypii* showed little variation in rate of growth over a range of pH 6.5 to pH 7.5.

(c) *Determination of nitrogen content.*

Since the recovery of nitrogen after hydrolysis is incomplete, it is essential to determine the nitrogen content of all hydrolysis products and to adjust it to the standard value. The determinations were carried out by the 'Micro-Kjeldahl' method outlined in Pregl's monograph (16). The distillation apparatus was that of Parnas and Wagner described by Pregl. As the method can be used with samples that contain 0.4-0.8 mg. of nitrogen, it is extremely valuable in cases where the amount of nitrogenous preparation available is very limited.

(d) *Experimental results.*

The amount of growth (dry weight) of *N. gossypii* on media containing peptone unhydrolysed or hydrolysed is given in Table II.

TABLE II.

Source of N.	No. of Plates.	Dry Weight of Yields (per 4 Plates) gm.
Peptone, Unhyd.	36	0.755 ± 0.014
" (Hyd. H ₂ SO ₄)	36	0.807 ± 0.017
" (Hyd. HCl; ppd, AgOH)	36	0.792 ± 0.006
" (" " neut. NaOH)	16	0.366 —
Control, no Peptone added	16	0.009 —

The medium without peptone allows of a thin starved type of growth. The source of nitrogen in this case is presumably impurities present in the agar, or possibly reserve nitrogen in the inoculum itself, which allows growth to continue for some time on the deficient medium.

It will be seen from the above Table that the amounts of growth obtained on the first three media were the same within the limits of experimental error. On the other hand the medium obtained by hydrolysis with hydrochloric acid and subsequent neutralization with sodium hydroxide was definitely inferior. That the reduced value of the latter was due to the presence of sodium chloride and not to any essential difference in the hydrolytic products obtained by the two acids was shown by a control experiment. The concentration of sodium chloride present in the fourth medium of Table II was known from the amount of alkali used in titration.

The control was prepared by adding an equivalent amount of sodium chloride to a medium in which the source of nitrogen was the product obtained with sulphuric acid. The amount of growth upon the control medium was 0.326 grm.

The above experiment, which was repeated four times in similar detail with like results, shows conclusively that *N. gossypii* does not require a complex molecule as the starting point of its nitrogenous metabolism but that it is able to build up its protein from simple amino-acids.

The same result was obtained for *Spermophthora gossypii*, *Nematospora coryli*, and the West Indian strain of *N. gossypii*.

B. Growth on Various Proteins.

The growth of *Nematospora gossypii* on certain other nitrogenous compounds was examined. The objects were to discover (1) whether the results obtained for peptone possessed any degree of generality for proteins, and (2) whether any correlation could be shown to exist between the amino-acid content of certain well-known proteins and their suitability as nutrients. Owing to their insolubility, proteins such as casein, gluten, and albumin (when heated in the autoclave) are difficult to disperse uniformly in a medium. An exact comparison of their relative nutritive values would therefore be difficult were it not for the fact that all of them in the unhydrolysed form proved to be valueless.

The sources of the various proteins tested were as follows :

- | | |
|-----------------|---|
| White of egg, | derived directly from fresh eggs. |
| Gluten, | prepared from wheat flour. The flour was mixed with sufficient water to make a stiff dough and allowed to stand for 15 minutes; then kneaded under running water until all the starch was washed out. The grey elastic residue was treated with several changes of acetone which removed the fat and also the moisture, thus bringing the gluten into a friable state. It was then ground to a fine powder in acetone under an end-runner mill. The acetone was finally allowed to evaporate. |
| Muscle protein, | prepared from ox muscle by boiling for several hours and subsequently extracting the solid with alcohol and ether to remove fats. The solid residue was dried and ground to a powder. |
| Gelatin, | ordinary sample as used in the preparation of culture media. |
| Lemco., | Commercial samples. |
| Casein, | |
| Edestin, | |

The method of hydrolysis and subsequent incorporation of hydrolysis products into a medium were exactly as described above for peptone.

The data obtained are given in Table III, the yields on unhydrolysed peptone being taken as 100 and those on other media calculated on this basis.

TABLE III.

Source of N.	Dry Weight of Yields.	
	On Unhydrolysed Protein.	On Hydrolysed Protein.
Peptone . .	100	101
Lemco . .	115	130
White of Egg .	negligible	100
Gluten . .	"	55
Casein . .	"	45
Muscle protein	"	34
Edestin . .	"	negligible
Gelatin . .	"	"

The protein substances examined fall into three groups.

(1) A group (peptone, 'lemco'), in which more or less equal growth is obtained whether the protein is hydrolysed or unhydrolysed.

(2) A group (white of egg, gluten, casein, muscle protein), in which the proteins themselves are unavailable, but which yield hydrolysis products which support growth in varying degrees.

(3) A group (gelatin, edestin) in which both the proteins and their hydrolysis products are valueless for growth.

The negligible growth on proteins of group (2) can readily be interpreted: one assumes that the fungus does not possess a proteolytic enzyme which renders the protein soluble. It is noteworthy that the members of group (1) (peptone, lemco), contain soluble products intermediate between proteins and amino-acids probably derived by partial hydrolysis of proteins. No group of substances was found in which the members were good nutrients as such and valueless after hydrolysis, i. e., no case has been found in which acid hydrolysis destroys the nutrient value of a nitrogenous organic compound for *N. gossypii*.

The growth yields obtained upon the hydrolysis products of the proteins in groups (2) and (3) have been considered with reference to the analyses of these proteins at present available in biochemical text-books e. g. Abderhalden (1), Plimmer (15), and Jordan Lloyd (10).

Muscle protein, casein, edestin, and white of egg are relatively complete and well balanced in amino-acids and on the supposition that amino-acids alone are concerned would be expected to give similar amounts of growth. Gluten is not typical in that it is low in bases and has a high dicarboxylic acid content. Gelatin lacks certain amino-acids, tryptophane, cystine, tyrosine, and is deficient in others. It is well known to have a very low nutritive value, and, according to Cathcart (5) it is a protein of peculiar physiological nature.

A study of the results shown in Table III does not indicate any clear correlation between the amino-acid content of the source of nitrogen and the amount of growth which it supports. There is no apparent reason why, if nutritive value depends simply on amino-acid content, the hydrolytic products of such proteins as casein, muscle protein and edestin should be inferior to those of white of egg. They all contain reasonably similar proportions of the known important amino-acids and bases. Further evidence on this point was obtained in the synthetic manner, as recorded in the following section.

C. Growth on Various Amino-acids and Mixtures of Amino-acids.

Experiments in this connexion were limited simply by the difficulty and expense of obtaining sufficient quantities of the various amino-acids. The question of the purity of commercial samples is also a matter of importance in tests of this description. A fairly representative selection was obtained, and after incorporation with the basal medium in a proportion corresponding to 0.3 per cent. nitrogen, tested as regards their nutritive value to *N. gossypii*. The following amino-acids and synthetic mixtures of amino-acids were used:

1. Glycine,
2. Alanine,
3. Leucine,
4. Aspartic Acid,
5. Glutamic Acid,
6. Phenylalanine,
7. Tyrosine,
8. Proline,
9. Tryptophane,
10. Cystine,
11. Mixture of Bases { Lysine,
Arginine,
Histidine, in the relative proportions 6:8:1.
12. 'Synthetic Protein', { Mono-Amino- { Glycine, 0.25 per cent.
with constituents ap- Acids { Alanine, 0.25 per cent.
proximately equivalent Leucine, 0.25 per cent.
to those of Lactalbu- Dicarboxylic { Glutamic Acid, 0.18 per cent.
min, according to the Acids. { Aspartic Acid, 0.18 per cent.
analyses of Jones and Cyclic { Proline, 0.13 per cent.
Johns (9). Compounds. { Phenylalanine, 0.05 per cent.
Tryptophane, 0.03 per cent.
Tyrosine, 0.05 per cent.
Protein Bases 0.67 per cent.

None of the media so prepared gave anything more than the negligible or starvation type of growth.

An additional experiment was carried out in this series to discover whether hydrolysed gelatin became a good nutrient when given a complete complement of amino-acids. Tryptophane (5 per cent.), tyrosine (5 per cent.), and cystine (1 per cent.) were added to hydrolysed gelatin.

TABLE IV.

Source of N.		Dry Weight of Yields.	
Hydrolysed Gelatin	.	.	negligible
"	+ 5 % Tryptophane	.	"
"	+ 5 % Tyrosine	.	"
"	+ 1 % Cystine	.	"
"	+ 5 % Tryptophane + 5 % Tyrosine + 1 % Cystine	}	"
Standard Peptone	.	.	0.334 gm.
"	+ 5 % Tryptophane	.	0.370 "
"	+ 5 % Tyrosine	.	0.379 "
"	+ 1 % Cystine	.	0.334 "

The presence of tryptophane, tyrosine, and cystine, separately or together, modified the starvation type of growth but did not increase it. The control experiment showed that the amino-acids added contained no inhibitory substances. It is therefore clear that hydrolysed gelatin does not supply the essential substances for the growth of the fungus even when supplemented with those amino-acids which it lacks.

D. Growth on Peptone Fractions.

A further series of experiments was carried out in which growth was measured on media containing (a) hydrolysed peptone, (b) two products of fractionation, and (c) a reconstituted mixture of the two fractions. The method of fractionation was as follows.

The bases of hydrolysed peptone were precipitated with 20 per cent. phosphotungstic acid in the presence of 5 per cent. sulphuric acid at 20° C. The filtrate, here termed Fraction A, contained the mono-amino-acids, together with the excess of phosphotungstic and sulphuric acids, which latter were removed with baryta in the usual way. The precipitate, which contains the phosphotungstates of the bases, was washed with 2 per cent. phosphotungstic acid, dissolved in a minimum quantity of acetone, decomposed with baryta, and filtered. The filtrate, here termed Fraction B, contains the bases. The pH values and nitrogen contents of the two fractions were then adjusted in the usual way. It may be added that the above method gave 66 per cent. recovery of both fractions.

To ensure that the chemical processes involved had left no deleterious residue in either fraction, a mixture of fractions A and B was reconstituted

in the proportions in which they were originally present. This necessitated a preliminary determination of the ratio of basic to total nitrogen in hydrolysed peptone. The bases were estimated by the micro-method of Thimann (19), the total nitrogen by Pregl's Micro-Kjeldahl method already cited. The ratio was found to be:

$$\frac{\text{Fraction B}}{\text{Fraction A}} = \frac{0.512}{1.713} = \frac{1}{3.4}.$$

In Table V are set out the results of an experiment in which the growth of *Nematospora gossypii* was measured on media containing these substances as sources of nitrogen (in all cases = 0.3 per cent. N).

TABLE V.

Source of N.	Dry Weight of Yield.
	gram.
Fraction A	0.221
Fraction B	0.264
Mixture of A and B (A : B = 3.4 : 1)	1.104
Orig. Hyd. Peptone	1.000

Thus, when either of the fractions was used alone, the amount of growth was reduced by about 75 per cent. That this result was not due to the presence of deleterious residues from the various reagents used in fractionization was shown by the fact that the reconstituted mixture of A and B supported growth as well as the original. Growth on Fraction B was of the staling type, i.e. the mycelial skin was not extremely thin, but it had not spread far over the medium. On the other hand, the growth on Fraction A was thin and widely spread, i.e. it was of the starvation type. The above results indicate that the substances which are important for growth have become separated between the two fractions, but the separation is not at all clearly marked, and the result cannot at the moment be definitely interpreted.

As a further attempt to elucidate the part played by these two fractions in nitrogen metabolism, each was combined with the complementary fraction of hydrolysed gelatin derived in exactly the same way. The combinations were made in the following proportions:

Fr. B from Peptone + Fr. A from Gelatin. (Ratio, 1/3.4).

Fr. B from Gelatin + Fr. A from Peptone. (Ratio, 1/3.4).

Control experiments were set up to show the growth due to the peptone fraction in each combination. The results are set out in Table VI.

It should be noted that in the medium (*b*) the amount of nitrogen present was less than a quarter of the standard (actually 1/4.4). On comparing the yields given by media (*a*) and (*b*) one sees that the A fraction of gelatin contributes very little. Similarly, from a comparison of media (*c*)

and (*d*) it is seen that though the B fraction of gelatin contributes less than a quarter of the total nitrogen of medium (*c*), nevertheless its presence multiplies the yield three times. Thus one sees that the B fractions are, per unit of nitrogen, the more important from the point of view of their effect on yield. Nevertheless, it is quite clear that neither the A nor the B fraction of gelatin replaces satisfactorily the corresponding fraction of hydrolysed peptone. The failure in particular of the B fraction of gelatin to replace the corresponding fraction of peptone was unexpected, seeing that gelatin as a protein is specially rich in the basic constituents. If it is true that amino-acids only are concerned in the problem of nitrogen metabolism, then one would expect the growth on (*c*) to be as good as that on (*e*). The fact that the results were otherwise and that no success was obtained in preparing a mixture of amino-acids which would be equal to hydrolysed peptone, appeared to indicate that some other substance or substances played a major part in the process of metabolism. The attempt at synthesis was necessarily incomplete as the full composition of peptone is unknown. It is possible that some amino-acid of prime importance was omitted from the mixture. On the other hand, it is unlikely that such an amino-acid would be present in peptone in large concentration, otherwise its existence would have been discovered. Thus one arrives at the same position as before, viz. that some substance, amino-acid or not, which is present in small amounts in peptone, plays an important part in nitrogen metabolism. This question will be taken up further in a later section.

TABLE VI.

Source of N.	Dry Weight of Yields.
	gm.
(<i>a</i>) B of Peptone + A of Gelatin	0.185
(<i>b</i>) B of Peptone (control to (<i>a</i>))	0.148
(<i>c</i>) B of Gelatin + A of Peptone .	0.233
(<i>d</i>) A of Peptone (Control to (<i>c</i>))	0.080
(<i>e</i>) Original Hyd. Peptone .	1.000

E. Assimilation in Presence of Peptone of Substances Otherwise Unavailable.

In the preceding sections it has been shown that growth is negligible when the source of nitrogen is asparagin or various amino-acids or hydrolysed gelatin. It was interesting to determine whether the fungus could assimilate nitrogen from such sources when peptone was present. Data bearing on this point are contained in Table VII and represented graphically in Text-fig. 2.

In the A media, the nitrogen content (in this case, peptone simply) varied from 25 per cent. to 100 per cent. of the standard amount (0.3 per cent. N). On the other hand, all the B and C media had the same total

nitrogen content (= 0.3 per cent. N), but this was partly made up of peptone (25 per cent., 50 per cent. . . &c.), and the remainder was asparagin or hydrolysed gelatin in the respective cases.

TABLE VII.

Media.	Concentration of Peptone (100 % = Standard = 0.3 % N).				
	25 %.	50 %.	75 %.	87.5 %.	100 %.
A. Peptone only . . .	0.118	0.364	0.668	0.864	1.042
B. " + Asparagin . .	0.468	0.900	1.130	1.260	"
C. " + Hyd. Gelatin .	0.459	0.914	1.124	1.205	"

The results clearly show that both asparagin and hydrolysed gelatin increase the amount of growth when added to a nutrient medium containing peptone. Whereas, with peptone as the sole source of nitrogen, reduction of the peptone causes a steady fall in the yield, the latter has not appreciably fallen when 50 per cent. of the peptone is replaced by otherwise unavailable sources. It would even appear that in certain proportions a mixture of peptone with asparagin or hydrolysed gelatin gives a higher yield than does a peptone medium of the same nitrogen content. Though the increases in these cases is not very great it has appeared twelve times, and one is therefore compelled to attach some significance to it.

Experiments were set up to determine whether the action of peptone just described is shown when the latter is present in minimal concentrations. The question was whether the action was of a catalytic nature or whether the magnitude of the effect produced was proportional to the concentration of peptone added. In one experiment two sets of media were prepared (1) with constant total nitrogen, but with peptone concentration varying from 0 per cent. to 50 per cent. of the standard amount, the remainder being hydrolysed gelatin, (2) controls with peptone only (0 to 50 per cent. of the standard nitrogen content). The data of yields (in mg.) are given in Table VIII.

TABLE VIII.

Medium.	Concentration of Peptone (100 % = Standard = 0.3 % N).							
	0 %.	2.5 %.	5 %.	7.5 %.	10 %.	12.5 %.	25 %.	50 %.
Peptone + Hyd. Gelatin	8	34	46	56	—	87	204	390
Peptone only . . .	4	10	17	21	28	31	56	104
Difference . . .	4	24	29	35	—	56	148	286

A similar experiment with asparagin replacing hydrolysed gelatin yielded comparable data. It was found, however, that the nitrogen of inorganic salts (ammonium sulphate, ammonium chloride, and sodium nitrate) was not assimilated even in the presence of high concentrations of peptone.

The results in Table VIII show that utilization of nitrogen from an 'unavailable' source is more or less proportional to the concentration of peptone in the medium, over a wide range. While traces of peptone induce

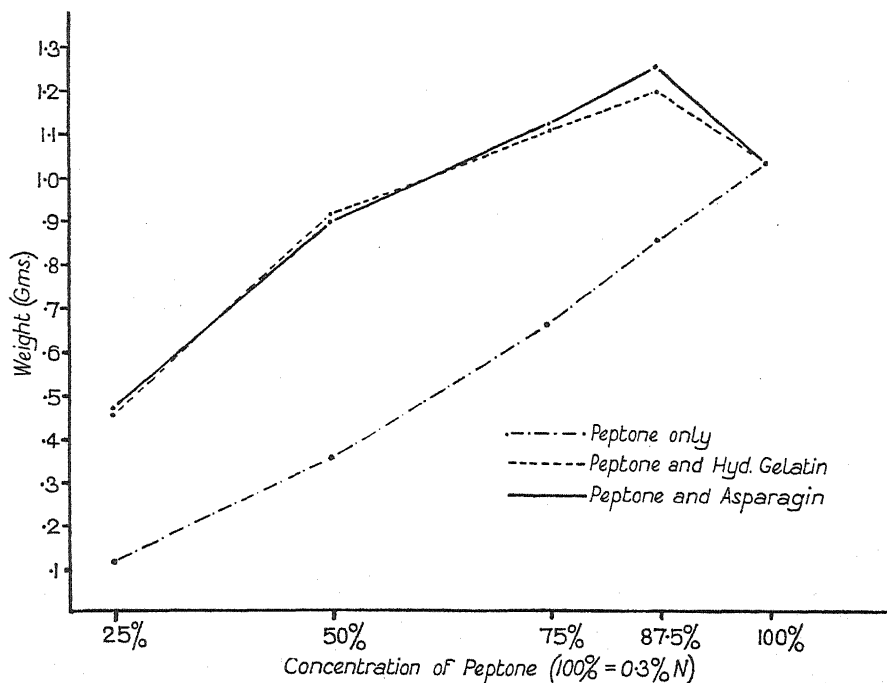


FIG. 2. Illustrating utilization of asparagin and hydrolysed gelatin in presence of peptone.

slight utilization it is clear that the effect is not catalytic, that is, small traces of peptone do not produce a relatively large assimilation of unavailable nitrogen compounds.

A comparative study of the data given in Tables VII and VIII will show that the amounts of growth obtained on media containing peptone were much less in the second case than the first. This was partly due to the shorter duration of the experiment of Table VIII (six days as against eight), but also to the use of a different sample of peptone. It has been noticed that different samples vary considerably both in their nutritive value when used alone and in their capacity to bring about the assimilation of a non-utilizable substance like hydrolysed gelatin.

F. Growth on Pure as against Impure Proteins.

The attempts to fractionate peptone described in Section D and to prepare a mixture of amino-acids which would be equivalent to a complete hydrolysed protein (Section C) on the whole led to negative results. In the

former no clear evidence of the nature of the growth promoting constituent was forthcoming, beyond the suggestion that it appeared to reside chiefly in the basic fraction. The failure of the attempts at synthesis led to the suspicion that an important element might be some substance, not necessarily amino-acid, which was present perhaps in very small concentration. Accordingly, experiments were carried out to test the behaviour of certain proteins which were more amenable than peptone to purification processes.

The first comparison was between white of egg and a crystalline albumin prepared by the method of salting out with ammonium sulphate described by Plimmer (*loc. cit.*, p. 434). Both were hydrolysed and prepared for incorporation in media in the usual way. The difference of growth obtained was very striking, the former giving results fully equal to that on standard peptone medium, and the latter giving negligible growth merely.

The preparation of a reasonable quantity of crystalline albumin involves great labour and expenditure of time. Experiments were therefore carried out to find whether comparable results could be obtained with an albumin of a lower grade of purity. The method adopted was as follows.

The white of thirty-six eggs (450 c.c.) was diluted with an equal volume of water, the fibres being thoroughly broken up by beating. This solution after filtration was slowly added to three times its volume of 95 per cent. alcohol, and again filtered, both filtrate and precipitate being retained. A portion of the latter was washed with alcohol and dried at 100° C. The remainder was taken up in water and reprecipitated three times, finally washed with alcohol and dried. Thus two samples of albumin were obtained, one of which was more thoroughly treated than the other.

The alcoholic filtrate and washings corresponding to each sample were evaporated to dryness, treated with absolute alcohol to precipitate the last traces of protein and filtered. The filtrate was freed from alcohol and made up to 500 c.c. of water. This liquid will be referred to as 'Egg Extract'.

The following four preparations of albumin were hydrolysed:—
(1) crude egg white, (2) and (3) the two samples mentioned above and
(4) a sample of crystalline albumin. The growth on these is shown in Table IX.

TABLE IX.

Source of N.	Dry Weight of Yields. gram.
Crude Egg White	0.915
Albumin (ppd. by Alcohol, unwashed) . . .	0.076
Albumin (" " washed three times)	0.012
Crystalline Albumin	0.007

It is thus seen that in the purification of egg white by alcoholic precipitation, a substance is lost which is essential for the nutrition of

Nematospora gossypii. With a single precipitation, and without washing of the precipitate, a product is obtained which gives a yield less than 10 per cent. of that given by the crude substance. When the precipitate is washed three times its growth-promoting capacity is still further reduced, and becomes in fact comparable to that of crystalline albumin. For experimental purposes, therefore, the laborious technique involved in the preparation of a crystalline albumin can be replaced by the simpler method outlined above.

The filtrate ('Egg Extract') was next examined as regards its capacity to support growth, both when used as sole source of nitrogen, and when added to the precipitated albumin in the proportion in which it was present in the crude material. The data are given in Table X.

TABLE X.

Source of N.	Percentage of N present.	Dry Weight of Yields. grm.
(a) 'Egg Extract' . . .	0.013	0.044
(b) Albumin	0.3	0.015
(c) 'Egg Extract' + Albumin	0.3	1.012
(d) Original White of Egg .	0.3	0.915

The growth on the medium (a) containing 'Egg Extract' only as source of nitrogen is small, but as the total percentage of nitrogen is very low, this would be expected in any case. The growth on albumin (b) is also small, though the content in nitrogen is standard. In (c) the 'Egg Extract' fraction is present in the same amount as in (a), so that it contributes less than one-twentieth of the total nitrogen. Nevertheless by its presence it raises the yield to fully the equivalent of that of egg white.

By simple alcoholic precipitation and subsequent washing, crude egg white can be split into two fractions, one of which (the precipitate) contains most of the nitrogen, but supports little growth; the other contains little nitrogen, but when added to the former fraction it completely restores the nutritive value.

(a) *Further examination of the 'egg extract'.*

Crude 'Egg Extract' (500 c.c. from thirty-six eggs) contained 1.68 per cent. total dry matter, and 0.013 per cent. nitrogen. It was subjected to various treatments in order to define more precisely the nature of the active substance present. The method employed was to break up the 'Egg Extract' into a number of fractions, the nutritive values of which were then tested by addition to the basal medium containing 0.3 per cent. N as hydrolysed pure albumin. Adjustment of the amounts of the various fractions to be added was difficult, particularly where chemical treatment involved loss of material. Total dry matter was used as the standard of

concentration, the percentage of nitrogen being too small and too variable to serve in this connexion. Such adjustment in comparative work was essential because, as shown in Table XI, the effect of the 'Egg Extract' was proportional to the amount present.

TABLE XI.

c.c. of 'Egg Extract' in 25 c.c. of Medium.	Dry Weight of Yields. gm.
0.25	0.093
0.5	0.127
1.0	0.170
4.0	0.260
8.0	0.348

That the active principle in the 'Egg Extract' is organic in nature, was proved by the following experiment.

The dry matter from 20 c.c. of extract was ignited, moistened with concentrated hydrochloric acid, and again evaporated to dryness. The residue was dissolved in water, and this solution incorporated in 100 c.c. of medium. 20 c.c. of the crude extract in 100 c.c. of medium served as control. The comparative yields are given in Table XII.

TABLE XII.

Medium.	Dry Weight of Yields. gm.
Basal + 0.3 % N + Ash of 'Egg Extract'	0.025
„ + „ + 'Egg Extract'.	1.440

Extractions with ether and alcohol were carried out as follows: 250 c.c. of 'Egg Extract' were shaken up with ether for five minutes, twice in acid and twice in alkaline solution. The total soluble matter was added, after removal of the ether, to 100 c.c. of medium. The ether-insoluble fraction will be referred to as Fraction A, the ether-soluble one as Fraction B. A continuous extraction for five hours gave a Fraction B¹ which was not materially different from B, either in amount or in growth-promoting capacity.

A portion of Fraction A was evaporated to dryness and extracted with absolute alcohol, giving an insoluble (C) and a soluble fraction (D). A portion of C was further extracted with 95 per cent. alcohol, giving Fractions E (insoluble) and F (soluble).

The various fractions were incorporated in 100 c.c. of the medium already referred to. The proportions of each were approximately as in 20 c.c. of egg extract, with the exception of B which, being of very small dry weight, was added in a quantity corresponding to 250 c.c. The yields obtained are given in Table XIII.

TABLE XIII.

Medium.					Dry Weight of Yields.
					gram.
Basal + 0.3 % N		+ Egg Extract			1.440
"	"	+ Fraction A			1.393
"	"	+	"	B	0.042
"	"	+	"	C	1.120
"	"	+	"	D	0.098
"	"	+	"	E	1.100
"	"	+	"	F	0.308

It is clear from the above figures that after successive extractions with ether, absolute alcohol and 95 per cent. alcohol, the bulk of the active substance remains in the insoluble residue. The fractions soluble in ether and absolute alcohol produce very feeble growth. On the other hand, it appears that greater activity is shown by the extract in 95 per cent. alcohol.

Fraction E which contains the bulk of the growth-promoting substance was further tested by a series of precipitation experiments. Three precipitants were used: copper hydroxide, lead hydroxide, and phosphotungstic acid.

Copper hydroxide method. A saturated solution of copper sulphate was added to a solution of Fraction E. Sufficient baryta was then added to precipitate the copper as the hydroxide and thereby carry down the sugars. The liquid was then filtered, the filtrate made slightly acid with sulphuric acid, and freed from copper and barium by passing hydrogen sulphide through the boiling liquid. It was then cleared with charcoal and evaporated to a volume calculated as equivalent to that of Fraction E, due allowance being made for loss. This gave Fraction H.

The precipitate was washed, boiled with water, and the copper compound decomposed by hydrogen sulphide in hot acid solution. After removal of the metallic sulphides by filtration, the liquid was cleared with charcoal and made up to the same volume as that of Fraction H. This gave Fraction G.

Fractions G and H had nitrogen contents equal to 0.33 per cent. and 0.46 per cent. respectively of the total dry matter. The former gave positive, the latter negative, results with Molisch's and Fehling's solutions. Fraction G thus contains all the sugars so far as these are demonstrable by the tests applied, and, it may be observed, an appreciable amount of the nitrogenous compounds.

Lead hydroxide method. The principle of this method is the same as that of the preceding. Lead hydroxide was precipitated from lead acetate and ammonium hydroxide in a solution of Fraction G. The precipitate was washed, redissolved in a minimum quantity of acetic acid, and reprecipitated

with ammonia. This precipitate was decomposed with hydrogen sulphide, freed from lead compounds, and the volume of the filtrate adjusted as in Fraction G. This is Fraction J. It gave reactions for sugars as before, and the nitrogen content was not reduced.

Phosphotungstic acid method. This was adopted in a further effort to free Fraction J from nitrogenous constituents. The method is the same as that described for the precipitation of the bases of peptone (p. 437). The precipitate obtained was negligible, even after three hours' standing. The filtrate after being freed from acids with baryta gave Fraction K. The nitrogen content of this was still much as before, being actually equal to 0.53 per cent. of the dry weight.¹

The yields obtained on media supplemented by these fractions are set out in Table XIV.

TABLE XIV.

Medium.	Dry Weight of Yields. gram.
Basal + 0.3 % N + Fraction G (20 c.c. in 100 c.c.)	0.976
" " + " H " "	0.014
" " + " J " "	0.877
" " + " K " "	0.709

From these results it is evident that the substance which promotes the growth of *Nematospora gossypii* is present in Fractions G, J, and K, but absent in Fraction H. That is, it is precipitated along with the sugars by copper hydroxide and lead hydroxide, but occurs in the filtrate after treatment with phosphotungstic acid. It is found, therefore, in a fraction which contains sugars, but is free from bases, and has a low nitrogen content.

A further series of experiments dealt with the oxidation of Fraction J (fraction precipitated by lead hydroxide). The oxidizing agents used were:—

- (1) Ammoniacal copper oxide.
- (2) Ammoniacal silver oxide.
- (3) Potassium permanganate.
- (4) Hydrogen peroxide.

(1) *Copper oxide method.* The reagent was prepared by adding excess sodium hydroxide to a solution of copper sulphate, and dissolving the precipitate, after washing, in a minimum quantity of ammonia. The ammoniacal solution was diluted to give an N/10 solution (approx.) of copper oxide.

¹ The amount of nitrogenous substance present in fractions G, H, J, and K is extremely low, and under these conditions Pregl's method does not give results of a high degree of accuracy. If the material had been available in large quantities, it could have been concentrated before analysis, and a better result thereby obtained. This, however, was not possible.

Before treatment with the reagent, Fraction J was boiled for fifteen hours with 5 per cent. sulphuric acid to hydrolyse any polysaccharides present. The sulphuric acid was then removed, and the solution made alkaline by addition of a few drops of concentrated ammonia.

The copper reagent was added drop by drop from a burette, and after the addition of each cubic centimeter, the liquid was boiled to assist decolorization. The end-point was reached when the solution remained permanently blue.

The ammonia was removed by adding a few drops of baryta, and boiling for ten minutes. The solution was made just acid with sulphuric acid and then treated with hydrogen sulphide to remove the copper. The precipitates (BaSO_4 and CuS) were finally filtered off, and the filtrate evaporated to a calculated volume. This product is Fraction L.

The copper reagent oxidizes the ketonic or aldehydic groups of the sugars, as well as aldehydic groups present in other compounds. That it does not oxidize (CH_2OH) or (CHOH) groups is shown by the fact that mannitol is not attacked. Thus the sugars are converted to sugar acids by this oxidation.

(2) *Ammoniacal silver oxide method.* The reagent was prepared from silver nitrate by the addition of excess sodium hydroxide. The precipitate, when washed free of alkali, was dissolved in a minimum quantity of concentrated ammonia, and diluted to give an N/5 ammoniacal solution of silver oxide.

Fraction J was hydrolysed as under (1) above, and a small quantity of charcoal was added to prevent the formation of a mirror by precipitation of silver during the oxidation.

The reagent was added, drop by drop, to a boiling solution of Fraction J. In this way the precipitate of silver was coagulated, leaving the supernatant liquid clear. The end point was reached when further addition of the reagent gave no cloudy precipitate. Ammonia and silver were removed as in (1) above. The product of this oxidation is Fraction M.

The silver reagent oxidizes the same groups as the copper reagent, and in addition it has been found to attack mannitol, and would therefore appear capable of converting the primary alcohol group (CH_2OH) to the carboxyl group (COOH). The product of this oxidation also is sugar acids.

(3) *Potassium permanganate method.* Fraction J was oxidized (a) with alkaline permanganate in the cold, as in Baeyer's 'Unsaturation Test', and (b) with acid permanganate in boiling solution.

(a) Baeyer's Test. In this test conditions limit the oxidation to the double bonds. Fraction J was diluted and made alkaline with sodium carbonate. Dilute potassium permanganate was added drop by drop until the liquid remained pink after standing for thirty seconds. To remove

manganese the solution was made alkaline with ammonia, then boiled, saturated with hydrogen sulphide, and cleared in the usual way. Ammonium sulphide was removed by boiling for thirty minutes. This gives Fraction N.

(b) Acid permanganate. Potassium permanganate was added to a boiling acidified solution of Fraction J until decolorization ceased. The solution was made alkaline with baryta to remove the sulphuric acid present. Manganese was removed as before in alkaline solution. Baryta was removed with sulphuric acid, and the solution boiled to remove hydrogen sulphide. This gives Fraction P.

Under such conditions larger quantities of potassium permanganate are reduced, and consequently appreciable amounts of potassium remain in solution. The oxidizing agent is extremely powerful and destroys sugars sugar alcohols, and unsaturated compounds.

(4) *Hydrogen peroxide method.* (a) Fraction J was treated with hydrogen peroxide for forty-eight hours at 20° C. in the presence of a trace of ferric chloride, as catalyst. Excess hydrogen peroxide was removed by prolonged boiling.

(b) Another portion of Fraction J was similarly treated with hydrogen peroxide in alkaline solution.

These processes give Fractions Q and R respectively.

The results of growth experiments using these oxidation products of Fraction J are set out in Table XV.

TABLE XV.

Medium.						Dry Weight of Yields.
						gm.
Basal + 0.3 % N	+	Fraction J	(20 c.c. in 100 c.c.)			0.877
"	"	+	" L "	"	"	0.600
"	"	+	" M "	"	"	0.906
"	"	+	" N (25 c.c. in 100 c.c.)			1.334
"	"	+	" P "	"	"	0.700
"	"	+	" Q "	"	"	0.126
"	"	+	" R "	"	"	0.581

The above Table shows that the growth-promoting substance is still active even after treatment of the solution with acid permanganate. The only oxidant that has largely destroyed its activity appears to be hydrogen peroxide in the presence of ferric chloride as catalyst. What significance is to be attached to the variations in activity shown in Table XV (apart from the obvious reduction in growth when Fraction Q is used) can only be determined by further work.

A series of experiments was carried out to test directly the possibility of the active substance being of the nature of a sugar or simple organic acid.

The active principle withstands hydrolysis with 22.5 per cent. sulphuric acid. In this process any polysaccharide would be split up to hexoses and pentoses, and the latter would be completely destroyed. Therefore if one tests the only four naturally occurring hexoses, viz. glucose, fructose, galactose and mannose, one exhausts the possibilities of the sugars.

Actually the percentage of sugars present in 'Egg Extract' was determined as being approximately 13 per cent. of the dry weight. A growth test was carried out on the following media :

- | | |
|-----|--|
| (1) | Basal + 0.3 per cent. N (pure Albumin) + 'Egg Extract' |
| (2) | " " " " + Glucose |
| (3) | " " " " + Fructose |
| (4) | " " " " + Galactose |
| (5) | " " " " + Mannose, |

the dry weights of sugar added being equal to the total dry weight of the egg extract (0.42 grm. in 100 c.c.) of the final medium. Growth in all the media with sugar only was negligible.

In a confirmatory experiment, glucose was oxidized by the ammoniacal copper oxide and silver oxide methods, and the oxidized products added to the medium in the usual way. Again the amount of growth was found to be negligible.

These experiments appear to exclude completely the possibility of the active substance being a sugar.

As certain organic acids would withstand the kind of treatment to which 'Egg Extract' was subjected, a trial was made with some of the commonly occurring organic acids along the same lines. The following acids were tested : malic, malonic ; succinic, lactic, glycollic, pyruvic, citric, and tartaric.¹

The results were as convincingly negative as with the sugars.

Further miscellaneous experiments which are relevant to the chemical nature of 'Egg Extract' are as follows :

(1) A standard nitration test gave no yellow colour, so that there is no aromatic derivative present.

(2) No precipitate was formed on addition of mercuric chloride, and only a slight indication of precipitation on addition of phosphotungstic acid. Therefore it appeared that bases were not present.

(3) The active principle withstood the hydrolytic action of boiling baryta (5 per cent.) for at least fifteen hours.

¹ Tartaric and citric acids do not resist the treatment described for 'Egg Extract', but are included for the sake of completeness. Pyruvic and lactic acids are of special interest, since it has been shown by Aubel, Genevois, and Salabartan (3) that the allied group, the yeasts, grow in synthetic media containing dextrose, lactic acid, or pyruvic acid.

(b) *Summarized conclusions as to the nature of the active substance.*

From the experiments described it is clear that the substance concerned is organic, soluble in water but insoluble in ether and absolute alcohol. It appears probable that it is an aliphatic compound, but not a polysaccharide, sugar, sugar alcohol, or sugar acid. Its insolubility in alcohol and ether shows that it is not a fat, ester, or lipid. The precipitation experiment with lead hydroxide points to the presence in the molecule of a (COOH) group, while the oxidation experiments do not exclude the presence of (CHOH) and (CH₂OH) groups. The substance appears to have properties not incompatible with those of an organic acid, but that it is not certain simple organic acids has already been shown.

The indications are that the active substance is a complex organic acid. Whether or not it contains nitrogen cannot be decided on the evidence available. If it is a nitrogen compound then it is certain that, as the amount of nitrogen added in the 'Egg Extract' is only 0.04 per cent. of that of the complete medium, the value of the active substance can have no relation to its being a source of nitrogen. That it is an amino-acid is highly unlikely in the light of the failure of growth on hydrolysed pure proteins. Furthermore, the precipitation experiments with mercuric chloride and phosphotungstic acid show that it is not a nitrogen base. Beyond this it is impossible at present to define more precisely the chemical nature of the active substance.

(c) *Preparation of a 'casein extract'.*

The experience with white of egg preparations indicated the possibility that similar results might be obtained with other naturally occurring proteins, e. g. casein of milk, which could conveniently be treated by the same methods.

The material used, which was concentrated separated milk, was treated practically in the same way as white of egg. The only important difference was that the alcoholic filtrate contained considerable quantities of salts which precipitated on evaporation. These were discarded. The casein so prepared was hydrolysed in the usual way. When growth was tested on the casein alone, and on the same after addition of 'Casein Extract', results were obtained as in Table XVI.

TABLE XVI.

Medium.	Dry Weight of Yields. gram.
Basal + Hyd. Casein (0.3 % N)	0.019
" " " + 'Casein Extract' (25 c.c. in 100)	1.144

It is clear from the above results that 'Casein Extract' contains a growth-promoting substance, and that casein which has been purified is of

little value as a source of nitrogen for the growth of *Nematospora gossypii*. A comparison of the results of Table XVI with those of Table III indicates further that the sample of commercial casein previously used was probably impure.

The properties of 'Casein Extract' were examined by the methods employed for 'Egg Extract', and it was found that precipitation with lead hydroxide gave a fraction J, which was as active as the original, and that the activity was reduced by oxidation with acid permanganate, and more markedly with hydrogen peroxide in the presence of ferric chloride. The active substance remained in the filtrate after precipitation with mercuric chloride or phosphotungstic acid, and was not destroyed by heating in an autoclave with normal sodium hydroxide for two hours at 140° C.

(d) *Assimilation of unavailable nitrogen compounds in presence of 'egg extract' or 'casein extract'.*

It has been shown that 'Casein Extract' induces the utilization of hydrolysed casein just as 'Egg Extract' induces utilization of hydrolysed albumin. It was interesting to test the effect of these two extracts on other unavailable sources of nitrogen.

The following were tested: asparagin, the hydrolysis products of gelatin, of casein, of albumin, and of pure fibrin. The sample of fibrin was prepared from horse's blood as described by Plimmer (loc. cit., p. 440), and its hydrolysis products were previously shown to be valueless as the sole source of nitrogen for *Nematospora gossypii*.

The results of the two series of experiments (a) with 'Egg Extract' and (b) with 'Casein Extract' are set out in Tables XVII and XVIII.

TABLE XVII.

Medium.		Dry Weight of Yields.
		gram.
Basal + 'Egg Extract' (Fraction J), 25 c.c. in 100 c.c.		0.030
"	" + Hyd. Gelatin	0.157
"	" + Asparagin	0.200
"	" + Hyd. Fibrin	0.406
"	" + Hyd. Casein	0.341
"	" + Hyd. Albumin	1.180

TABLE XVIII.

Medium.		Dry Weight of Yields.
		gram.
Basal + 'Casein Extract' (Fraction J) 25 c.c. in 100		—
"	" " + Hyd. Gelatin	0.276
"	" " + Asparagin	0.280
"	" " + Hyd. Fibrin	0.521
"	" " + Hyd. Casein	1.183
"	" " + Hyd. Albumin	1.193

The above results show that the two extracts stimulate growth on nitrogenous substances other than their own associated proteins. The growth so obtained is, however (with the possible exception of the combination 'Casein Extract' + Hyd. Albumin) less than when the extract is added to its own protein, and therefore some degree of specificity is suggested.

The 'Casein Extract' used appears to be more active than the 'Egg Extract' as is shown by a comparison of the corresponding figures of Tables XVII and XVIII, but whether any significance is to be attached to such quantitative comparisons can only be shown by further work. Obviously in such work the question of standardization of the extracts used would require first consideration.

V. COMPARISON OF THE GROWTH-PROMOTING SUBSTANCE OF *NEMATOSPORA GOSSYPII* WITH SIMILAR SUBSTANCES DESCRIBED ELSEWHERE.

The main result arising from the preceding account is that the fungus *N. gossypii* is unable to assimilate the nitrogen of a pure protein, whereas in the presence of some complementary substance it may freely do so. There remains now to discuss some analogies between the results here recorded and similar ones which have been described in other connexions.

Hopkins (8) and various other workers have shown that in animal nutrition proteins are assimilated in the form of their constituent amino-acids. In this respect, and also in the non-utilization of gelatin, the behaviour of *N. gossypii* is strictly parallel. Beyond this point the analogy ceases. The animal organism, according to Kaufmann (11), assimilates gelatin to a certain extent when the amino-acid tryptophane is added. Both by direct test, and by the fact that tryptophane cannot resist the method of hydrolysis adopted, it is shown in this paper that that amino-acid possesses no particular significance in the metabolism of *N. gossypii*. The growth-promoting factor in this case is probably not an amino-acid, nor even a nitrogenous compound at all.

Closer analogies are offered by the water-soluble vitamin B, by the 'Bios' substance of Wildiers (20), and more especially by a bacterial growth-promoting substance described by Reader (17).

It appears from the work of Kinnersley and Peters (12) that vitamin B is much less resistant to heating with alkali than the growth-promoting substance here described. In its behaviour towards oxidizing agents, it shows a general similarity. On the crucial question as to whether there is any similarity in physiological behaviour, there is at present no information available.

Comparison with the 'Bios' substance of Wildiers is difficult on account of the extremely controversial statements as to the properties, and even the

existence of that substance (Tanner, 18). So far as one can gather, 'Bios' is a substance the presence of which is supposed to be necessary for the growth of yeasts on a mineral nutrient. Whether it is manufactured by the yeast cells, once they have begun to grow, or whether it is merely carried over from a previous nutrient medium through the inoculum, is a disputed point. There is no suggestion in the present work that the growth-promoting substance is produced by *N. gossypii*, nor that its effect is catalytic. The analogy with 'Bios' is thus lessened if it is claimed that the latter is of an autocatalytic nature. If 'Bios' is merely an impurity derived from a preceding culture a closer analogy is possible.

The closest analogy is found in a bacterial growth-promoting substance described by Reader (loc. cit.). This substance is present in various natural extracts, and has a function quite similar to that of the growth-promoting substance of this paper. Its properties as given by Reader indicate further resemblances. Thus it shows similar solubility relationships, and in particular it is not precipitated by phosphotungstic acid. It differs from the growth-promoting substance of *N. gossypii* in showing a less degree of resistance to alkalis, and apparently also in its reaction towards mercuric chloride as a precipitant. What importance is to be attached to such differences, and how far they are merely conditional on the presence of other substances as impurities in the preparations tested, requires further investigation. It is clear, however, that there are very strong resemblances in mode of occurrence, properties, and physiological function.

We wish to record our thanks to Prof. W. Brown, who outlined the problem to us in the first instance, and under whose direction we have worked. As regards the more chemical aspects of the work we are indebted to Prof. Schryver for facilities offered in his Department. For instructions and help in carrying out many of the chemical processes and estimations, we wish to thank Dr. Buston, Dr. Hand, and Mr. Town, of the Biochemical Department.

VI. SUMMARY.

1. The fungi *Spermophthora gossypii*, *Nematospora coryli*, and *Nematospora gossypii* (two strains), are somewhat selective as regards their source of carbon food supply. They have no power of fermenting sugary liquids. In this respect they differ strikingly from the yeasts, a group to which they are considered to be allied.

2. These fungi do not grow on a medium in which the source of nitrogen is asparagin, but grow freely when nitrogen is added in such forms as peptone or 'lemco'.

3. A complex nitrogenous molecule is not required as such, as shown by the fact that growth takes place on a medium in which the source of nitrogen is completely hydrolysed to its constituent amino-acids (e.g. peptone,

'lemco', white of egg and gluten, all hydrolysed). Certain proteins such as gelatin, edestin, and pure fibrin are valueless whether hydrolysed or unhydrolysed.

4. While there is good growth on peptone and various hydrolysed proteins there is only a negligible amount on a mixture of amino-acids which is representative as far as possible of the hydrolytic products of a complete protein.

5. Substances such as asparagin and hydrolysed gelatin which are unavailable by themselves as source of nitrogen are assimilated in the presence of peptone. The action of peptone in such cases is quantitative and not catalytic. Inorganic salts of nitrogen are not assimilated in presence of peptone.

6. The utilization of the protein of white of egg is conditional on the presence of a growth-promoting substance from which the protein is readily separable by alcoholic precipitation. A similar behaviour is shown in the case of milk protein. In addition the active extracts prepared from white of egg and from milk induce some degree of assimilation of such unavailable sources of nitrogen as asparagin, hydrolysed gelatin and hydrolysed fibrin.

7. From a study of the chemical properties of the growth-promoting substance, it is suggested that the latter is probably of the nature of an organic acid. The active substance corresponding to each protein is probably to a certain extent specific.

8. A discussion is given of the analogy presented between the growth-promoting substance described in this paper and such substances as Vitamin B, 'Bios', and a substance which stimulates the growth of a *Streptothrix*.

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Studies in the Inheritance of Physiological Characters.

I. A Physiological Investigation of the Nature of Hybrid Vigour in Maize.

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With Plate XXIV and one Figure in the Text.

I. INTRODUCTION.

IT has long been known that the continued inbreeding of many animals and plants produces weakened races, and that the first hybrid cross from inbred parents is often remarkably more vigorous than either parent. This phenomenon, which has been called *heterosis* (5), has been extensively investigated in maize, in which plant it is strikingly manifested. The investigations, however, have been concerned principally with the genetics of heterosis, and with the effect of hybridization upon yield. As far as the writer can gather, observations upon the physiological differences between parent and hybrid have been more or less incidental.

Nothing definite is known as to the primary cause of hybrid vigour. Two theories have been put forward. It has been suggested by some writers (3, 8) that fertilization by a 'foreign' sperm 'accelerates the interaction between nucleus and cytoplasm' (Shull, 8). This acts as a kind of stimulus on the whole development of the organism. On the other hand Jones (6) has put forward the view that the vigour of hybrids is due to a combination of factors from both parents, favourable to growth, and that continued inbreeding masks these factors. Toward this problem plant physiology has little to contribute; the physiology of fertilization and cell division is a subject yet to be developed. But to the more immediate causes of vigour, to the question 'how is the greater vigour of the hybrid manifested?', plant physiology can supply some answer.

The increased size of the hybrid over its parents might be due simply

to the fact that its somatic cells are bigger than those of the parents. This, however, is not the case. Now hybrid vigour in maize might be manifest in four ways: 1. More meristematic centres may be present in the hybrid, giving more leaves and tillers. 2. The leaves of the hybrid may be more efficient in photosynthesis. 3. The embryo may be bigger, so that the capital with which the hybrid germinates is bigger. 4. Finally, the falling off in the sigmoid curve of growth might occur much later in the season for the hybrid than for its parents; in the event of this being the explanation, any difference in growth due to hybrid vigour would only be apparent late in the season.

Although previous workers have recorded observations upon some of these points, it has never been made clear which of the above possibilities, or which combination of them, is immediately responsible for the hybrid vigour in maize.

East and Hayes state (4) that the crossing of inbred strains affects 'the amount and rapidity of assimilation as expressed by cell division'. It is uncertain what the authors mean by 'assimilation' here; it is uncertain, too, whether the increased cell-division is the result of, or results in, increased assimilation. In the same paper the authors mention increased height, (entirely internodal), tendency to early maturity, and a slight increase of cotyledon size, as phenomena which accompany heterosis. In a more recent work (5), East and Jones mention increased germinative capacity of the hybrid seeds, and a heavier embryo and endosperm. Finally, Castle (2) in 1926 found that when tumours are grafted into the parents of an inbred strain of mice, and into the first hybrid generation, the tumours grew more rapidly in the hybrids. He suggests that heterosis causes the acceleration of *all* the metabolic processes in the hybrid.

It is apparent that there has been no clear statement as to what physiological processes are affected in heterosis, and what are not. The investigation to be described is intended to throw some light on this aspect of hybrid vigour, as it is found in maize.

II. MATERIAL.

Three cobs of maize were obtained from the United States Bureau of Plant Industry, Washington.¹ These were described as:

1. Strain 239, known in this paper as P_w .
2. " 281, " " " P_b .
3. " 237, " " " F_1 .

P_w was an inbred starchy seed, white in colour. P_b was an inbred blue variety, as used by North American Indians. The seeds F_1 were light blue in colour, and were the result of the first cross between P_w and P_b .

¹ I am indebted to Dr. Collins of the U.S. Bureau of Plant Industry for this material.

As a preliminary the seeds were weighed. There was found to be very little variation in any of them. The mean weights of fifty seeds were as follows:

P_w	0.368	gram.	0.010
P_b	0.163	„	0.011
F_1	0.405	„	0.009.

It will be seen that the seeds of the hybrid are scarcely significantly heavier than those of the white parent, though they are considerably heavier than those of the blue parent. Since the greater part of the seed, however, is stored food, and not active tissue, the embryos themselves were dissected out and weighed at a later stage in the work. The results are discussed on pages 463 and 464.

III. FIELD EXPERIMENT.

In order to observe the growth of the three strains of maize under natural conditions, a field experiment was begun at Slough, Bucks, in May 1929, in a uniform plot of light loamy soil. Three seeds were sown together in 'hills' three feet apart. The rows of hills were two feet apart. The order of sowing was in blocks of rows as follows:

row:	1	2	3	4	5	6	7	8	9	10	11	12,	&c.
	P_w	F_1	P_b	F_1	P_b	P_w	P_b	P_w	F_1	P_w	F_1	P_b	
	

When the seeds had germinated, all but one seedling was removed from each hill. There remained about eighty plants of each strain. This provided for samples of ten every fortnight for sixteen weeks.

For sampling, ten plants of each strain were dug up, and the total leaf area and dry weight of each plant found. The leaf area during the first thirty-three days of growth was found by drawing the outlines of each leaf on paper, and measuring the area with a planimeter. For the fourth sampling this method proved to be impracticable, owing to the large number and size of the leaves; accordingly the length and greatest breadth of the leaves were taken, and the areas of a few of them found with a planimeter. In this way an empirical relationship was established between area and the product of length and breadth, and from this relationship the approximate area of the other leaves could be found. The standard error did not exceed 9 per cent. For the dry weight estimations the plants were dried in an electric oven at 100° C. for fifteen hours. In addition to the dry weight and leaf area estimations, comparable leaves, one from one plant of each sample, were employed for estimations of the respiration and assimilation by the colorimetric method described by Bolas (1).

The seeds were sown on 4 May 1929. The hybrid plants germinated

shortly before the parents. After three weeks, when the first sampling was carried out, there were many obvious differences between the hybrid and the parent plants, in size, in appearance, and in number of leaves. It was also noticed that the percentage germination of the three strains was very different, as the following figures show:

Strain	P_w	P_b	F_1
per cent. germination	47	30	90

The germination of the hybrid exceeds the average germination of the parents by 51 per cent. In characters such as leaf number and tiller number the hybrid resembles the parent P_b throughout the experiment.

In Table I are given the results of the first sampling in full. Table II contains the assimilation and respiration of individual leaves from the three strains for the first and second samples.

TABLE I.

Plant No.	Area sq. in.	P_w Strain.		P_b Strain.			F_1 Strain.		
		Dry Weight in grm.	Weight Area.	Area sq. in.	Dry Weight in grm.	Weight Area.	Area sq. in.	Dry Weight in grm.	Weight Area.
1	3.88	0.221	0.057	1.76	0.119	0.068	7.06	0.443	0.063
2	2.64	0.235	0.089	0.93	0.080	0.086	7.05	0.343	0.049
3	4.31	0.299	0.070	3.92	0.188	0.048	7.48	0.461	0.062
4	3.13	0.298	0.095	1.13	0.056	0.049	7.87	0.418	0.053
5	2.89	0.255	0.088	3.13	0.186	0.059	8.49	0.459	0.054
6	3.52	—	—	2.95	0.147	0.050	10.37	0.443	0.044
7	3.05	0.283	0.093	1.25	0.128	0.100	7.40	0.321	0.043
8	3.04	0.262	0.086	1.50	0.109	0.073	4.68	0.323	0.069
9	3.35	0.231	0.069	2.25	0.155	0.069	8.84	0.432	0.049
10	3.55	0.215	0.064	3.02	0.152	0.050	8.04	0.485	0.060
mean	3.32	0.255	0.078	2.18	0.132	0.061	7.73	0.413	0.054
σ	0.32	0.032	—	0.94	0.043	—	1.22	0.058	—

Sampling: 21st day of experiment.

TABLE II.

	21st Day.	Youngest fully open Leaf.		33rd Day.	Youngest fully open Leaf.	
	Respiration Weight.	Apparent Assimilation Weight.	Assimilation Respiration.	Respiration Weight.	Apparent Assimilation Weight.	Assimilation Respiration.
P_w	3.95	0.265	4.9	2.78	0.14	5.2
P_b	3.50	0.270	5.0	—	—	—
F_1	2.90	0.212	5.1	2.75	0.16	4.4

The figures in Table I show that after three weeks' growth the average dry weight and area of the hybrid plants are significantly bigger than the dry weight and area of either of the parents. The values for weight per unit area, which do not differ from one another significantly, suggest that

the leaves of the hybrid (which unfolded at the same time as the leaves of the *b* Strain) are no more efficient in photosynthesis than the leaves of the parent plants. This suggestion is confirmed by the values for the assimilation-respiration ratio obtained from comparable leaves from the three strains (Table II). In Table III is set out a condensed statement of the results of the whole experiment.

TABLE III.

Time in Days.	Strain.	Mean Weight.	σ	Mean Area.	Weight Area.	Mean No. of Leaves.	Mean No. of Tillers.	
0	<i>P_w</i>	0.370	—	—	<i>a</i>	—	—	} These values are the mean weights of the seeds.
	<i>P_b</i>	0.163	—	—	<i>a</i>	—	—	
	<i>F₁</i>	0.405	—	—	<i>a</i>	—	—	
21	<i>P_w</i>	0.255	0.032	3.32	0.079	3	—	
	<i>P_b</i>	0.132	0.043	2.18	0.056	4	—	
	<i>F₁</i>	0.413	0.058	7.73	0.055	4	—	
33	<i>P_w</i>	0.494	0.064	10.10	0.049	5	—	
	<i>P_b</i>	0.344	0.055	6.75	0.051	6	—	
	<i>F₁</i>	1.466	0.121	28.8	0.051	6	—	
44 ¹	<i>P_w</i>	1.21	0.182	24.0	0.05	6-7	1	
	<i>P_b</i>	1.36	0.167	36.0	0.04	10-12	2	
	<i>F₁</i>	3.95	0.69	88.0	0.05	12-14	2	
59	<i>P_w</i>	3.3	0.29	—	—	—	1-2	
	<i>P_b</i>	4.0	0.37	—	—	—	3	
	<i>F</i>	16.3	1.7	—	—	—	3	
73	<i>P</i>	7.6	0.77	—	—	—	1-2-3	
	<i>P_b</i>	17.4	2.0	—	—	—	3-4	
	<i>F₁</i>	66.0	6.9	—	—	—	3-4	
83	<i>P_w</i>	10.9	—	—	—	—	—	
	<i>P_b</i>	27.5	—	—	—	—	—	
	<i>F₁</i>	97.7	—	—	—	—	—	

Throughout the whole experiment the hybrid plants were taller and heavier than either of the parents, and their constituent organs were on a bigger scale. After seventy-three days *F₁* is nine times as heavy as *P_b* and twice as heavy as *P_w*. The initial loss in weight of all the plants is due to the fact that the germinating seedling lives on capital until it begins to assimilate carbon dioxide for itself. The strain *P_b* seemed feeble at the beginning, but subsequently it formed tillers, and thereafter grew bigger than Strain *P_w*, on which fewer tillers had formed. Leaf areas were taken until the forty-sixth day of the experiment, and throughout this time the ratio of dry weight formed to leaf area remained about the same. This indicates that the leaves of the hybrid are relatively no more efficient in 'earning capacity' than the leaves of either of the parents. The suggestion is confirmed by a second series of determinations of assimilation and respiration in the laboratory after thirty-three days, the results of which have been given in Table II. The figures in Table II are taken from

¹ Photographs of typical individuals from the three samples are reproduced in Plate I.

determinations on single leaves, but it seems reasonable to conclude that there is no striking difference between the assimilation-respiration ratio of the leaves.

IV. GROWTH CURVES OF THE FIELD EXPERIMENT.

When the average dry weights of the plants in each strain are plotted against time, the values, after an initial drop lie upon an exponential curve for the duration of the experiment, with the exception of those values after eighty-five days which fall below the curve. It can therefore be concluded that the characteristic flattening of the sigmoid curve has begun at this point. It is significant that it has begun in all three strains at the same time.¹

In the text-figure the logarithms of dry weight are plotted against time. It will be seen how closely the values fall upon a straight line. Equations of closest fit to these lines have been calculated. These are given in Table IV, together with the calculated and observed values of the dry weight. The agreement is satisfactory throughout.

TABLE IV.

Growth Curves from 21st day to 73rd day.

Dry Weight.						
$P_w. W = 0.0525 e^{0.0692t}$		$P_b. W = 0.0155 e^{0.0965t}$		$F_1. W = 0.0595 e^{0.0956t}$		
t.	cal.	obs.	cal.	obs.	cal.	obs.
0	0.0525	—	0.0155	—	0.0595	—
21	0.224	0.244	0.123	0.132	0.452	0.413
33	0.502	0.494	0.38	0.344	1.445	1.466
44	1.1	1.21	1.16	1.36	4.075	3.95
59	3.1	3.3	4.7	4.0	16.9	16.3
73	7.92	7.6	16.8	17.4	64.6	66.0

The most striking fact displayed by the text-figure is that *the relative growth rates of the hybrid strain and the parent strain P_b are identical*. The straight lines represented by the equations $y = 0.0415x + 1.215$, and $y = 0.0419x + 1.789$ are, within the limits of experimental error, parallel. The vigour of the hybrid, then, is *not* due to an increased relative growth rate over this period of the life-cycle. As regards its relative growth rate the hybrid generation cannot be considered any more vigorous than its parent P_b ; it only differs from its parent in starting germination with a greater *capital*.

The next step in the investigation was to find the nature of this greater capital. According to the values for zero time calculated in Table IV, the relative weights of the embryos at the beginning of the experiment should be in the ratio $P_b : P_w : F_1 :: 1.0 : 3.4 : 3.8$. To test this, seeds of the three

¹ The climatic conditions of the week preceding this sampling were not unfavourable in any way.

strains were soaked, and after forty hours the embryos were dissected out from the endosperm. The scutellum was removed from each embryo, and

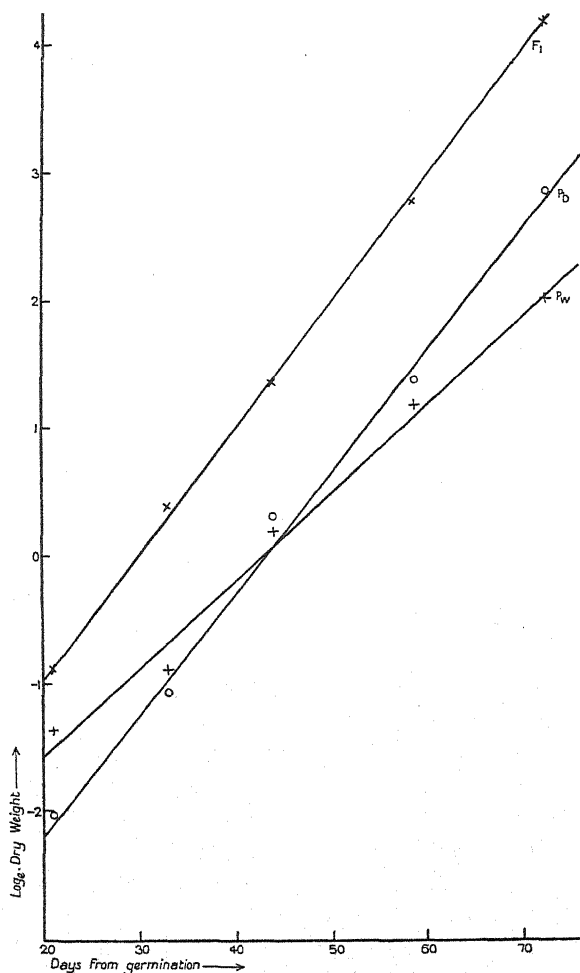


FIG. 1.

the embryos were weighed. Both wet and dry weights were found. The following table (Table V) gives the results of these weighings:

TABLE V.

	Wet Weight. ¹	Dry Weight. ¹	% H ₂ O.	Dry Weight Ratio.
<i>P_w</i>	0.099	0.038	65	3.1
<i>P_b</i>	0.035	0.0125	64	1.0
<i>F₁</i>	0.125	0.045	64	3.6

¹ Mean of eight embryos.

The dry weights of the embryos, when compared with those values calculated for zero time in Table IV, will be seen to be somewhat low. But the embryos which were weighed had already germinated for forty hours, and had lost weight through respiration throughout that time, though this does not altogether account for the discrepancy. What is important, however, is that the ratio of the weights of the three embryos is of the same order as the ratio calculated from Table IV, viz. 1 : 3.4 : 3.8.

V. CELL SIZE.

It remained to be discovered whether the heavier embryo of the hybrid strain contained more cells than its parents, or whether the cells were bigger; also to gain some idea of the relative amounts of respiring material in the embryos of the three strains.

Sections of plumules of three-day old seedlings were cut and cell counts were made, both longitudinally and transversely under the microscope, with a graduated eyepiece. The average counts obtained were as follows:

Number of Eyepiece Divisions over Ten Cells.
(Mean of five readings.)

	Transversely.	Longitudinally.
P_w	7.5	14.2
P_b	8.3	13.7
F_1	7.8	13.8

The length and breadth of the cortical cells of all three strains were found to be about the same. The cells of the hybrid, then, are not noticeably bigger than the cells in the plumules of either parent.

Estimation, by the colorimetric method, of the respiration of embryos three days old suggested that there was no wide difference between the respiration of P_b and F_1 , while both had a slightly higher respiration than P_w . The results, however, are not very trustworthy, and are being repeated.

It appears, then, that the embryo of the hybrid strain contains more cells than either of the parents. Once it has germinated, its relative growth rate is no greater than that of the parent P_b , nor does its respiration seem to be significantly different.

VI. CONCLUSIONS.

If the four possible ways in which hybrid vigour might be manifest, suggested on p. 458, be considered in the light of these results, the following conclusions can be drawn:

Hybrid vigour in maize is not due to the presence of a greater number of meristematic centres in the hybrid, since for the greater part of the

experiment the number of leaves and tillers in F_1 equalled the number in P_b . It is not due to an increased size of the somatic cells nor to an increased photosynthetic efficiency of the leaves. Finally, indications have been given that the flattening of the sigmoid curve of dry-weight-increase \times time occurs at the same time for all three varieties, so that none of the vigour can be ascribed to a longer 'grand period' of growth.

The vigour of the hybrid is apparent to the eye directly after germination, and is maintained throughout the growing period. The *relative* growth rate of the hybrid, though bigger than that of one parent, is exactly the same as that of the other parent. In fact, apart from the higher percentage germination, the only fundamental difference which has been discovered between the hybrid and its parent P_b is that *the hybrid embryo is larger and heavier than that of the parent, and so germinates with an initial advantage which is retained throughout the life-cycle*. In some process between fertilization and the setting of seed lies the immediate cause of hybrid vigour. After germination the hybrid is no more 'vigorous', as measured by activity of physiological processes, than its parent P_b .

VII. THE INHERITANCE OF GROWTH RATE.

An inspection of the growth curves reveals one other interesting fact. From the text-figure it is evident that the relative growth rate, as expressed by the coefficient of x , 0.0415, is exactly the same as that of parent P_b , and is apparently quite uninfluenced by parent P_w . In other words, the relative growth rate is not greater than that of the parents, nor intermediate between them, *but is apparently inherited from one parent in the manner of a dominant Mendelian character*. Whether relative growth rate is in fact governed by a process which is a simple Mendelian character cannot be declared with certainty without an analysis of the F_2 generation. It is hoped that such analysis will shortly be carried out.

While it is true that such a factor as relative growth rate may be regarded as the resultant of a number of more or less independent processes, its very constancy is an index of its importance as a measure of the 'economic efficiency' of the plant, and the mode of inheritance of 'economic efficiency' is really more important than the inheritance of its constituent processes.

These results open up the question of the inheritance of physiological characters. Many morphological characters, the genetics of which are admittedly easy to analyse, throw no light whatever upon the efficiency of the plant in growth and competition with other plants. Moreover, since many morphological characters play no part in the struggle for existence, a study of their inheritance throws no light upon the problems of adaptation and evolution. Upon its physiological characters, viability of seed, photosynthetic efficiency, resistance to drought, depends the success or failure of

a plant, and a study of the inheritance of these characters may be expected to furnish results of greater practical value and theoretical interest.¹

I wish to record my gratitude for the kindly encouragement of Professor V. H. Blackman. I have also to acknowledge my indebtedness to Miss M. H. Collett-Brown for her assistance in the routine work of sampling, and to Dr. P. H. Hicks for carrying out the respiration and assimilation determinations.

VIII. SUMMARY.

The primary object of the work described is the discovery of the physiological differences between a first hybrid generation of maize, and the two inbred parents from which it was derived, and to ascertain whether these differences can account for the phenomena of hybrid vigour.

Two strains of maize, which had been inbred for some years, were grown, together with the first cross between them. Directly the plants were above ground the characteristic vigour of the hybrid was visible, and the hybrid plants were bigger than either parent throughout the experiment. Samples were taken at regular intervals, and the mean dry weight and total leaf area per plant found, together with the assimilation and respiration rates of comparable leaves.

It was found that *the hybrid does not differ in the least from its more vigorous parent as regards relative growth rate*, nor does it differ from either of its parents as regards cell size, photosynthetic efficiency of leaves, or the time of flattening of the sigmoid curve of growth.

The only physiological differences observed were an increased percentage germination on the part of the hybrid, and also a *greater initial weight of the embryo, which gave an advantage which was maintained throughout the grand period of growth*.

The phenomenon of hybrid vigour is due to some process between fertilization and the setting of seed. After germination there is no real difference between the growth of the hybrid and that of the more vigorous parent.

The results bring to light one other fact: that the *relative growth rate is apparently inherited in the manner of a dominant Mendelian factor*. The exact mode of inheritance is to be the subject of further study.

ADDENDUM.

'A recent paper by R. C. Robb (British Journal of Experimental Biology, vi, 1929, pp. 293-310) presents data for rabbits somewhat similar to the data here presented for maize. Flemish Giants were crossed with

¹ As an example of such investigations may be quoted the work of Pearl and his collaborators (7).

Polish rabbits, and the growth curves of parents and offspring were fitted to curves of the form :

$$t = \frac{1}{K_1 + K_2 A} \log \frac{A(K_2 x + K_1)}{K_1(A - x)}.$$

This equation is the modification of Robertson's equation, suggested by Crozier (Journal of Gen. Phys., x, pp. 53-73, 1926-7). K_1 represents the velocity constant of a first order reaction, and K_2 that of a reaction catalysed by x , the product of the reaction. In size the hybrid offspring were intermediate between the two parents, but the values of K_1 for the hybrid were practically *identical* with the values for the Polish parent, and about double the values for the Flemish Giant parent.'

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EXPLANATION OF PLATE XXIV.

Illustrating Mr. Ashby's paper on the Nature of Hybrid Vigour in Maize.

The photograph illustrates three typical plants, one from each strain, after 44 days' growth. The hybrid F_1 inherits the characters of leaf number, tiller number, and relative growth rate from the parent P_2 .



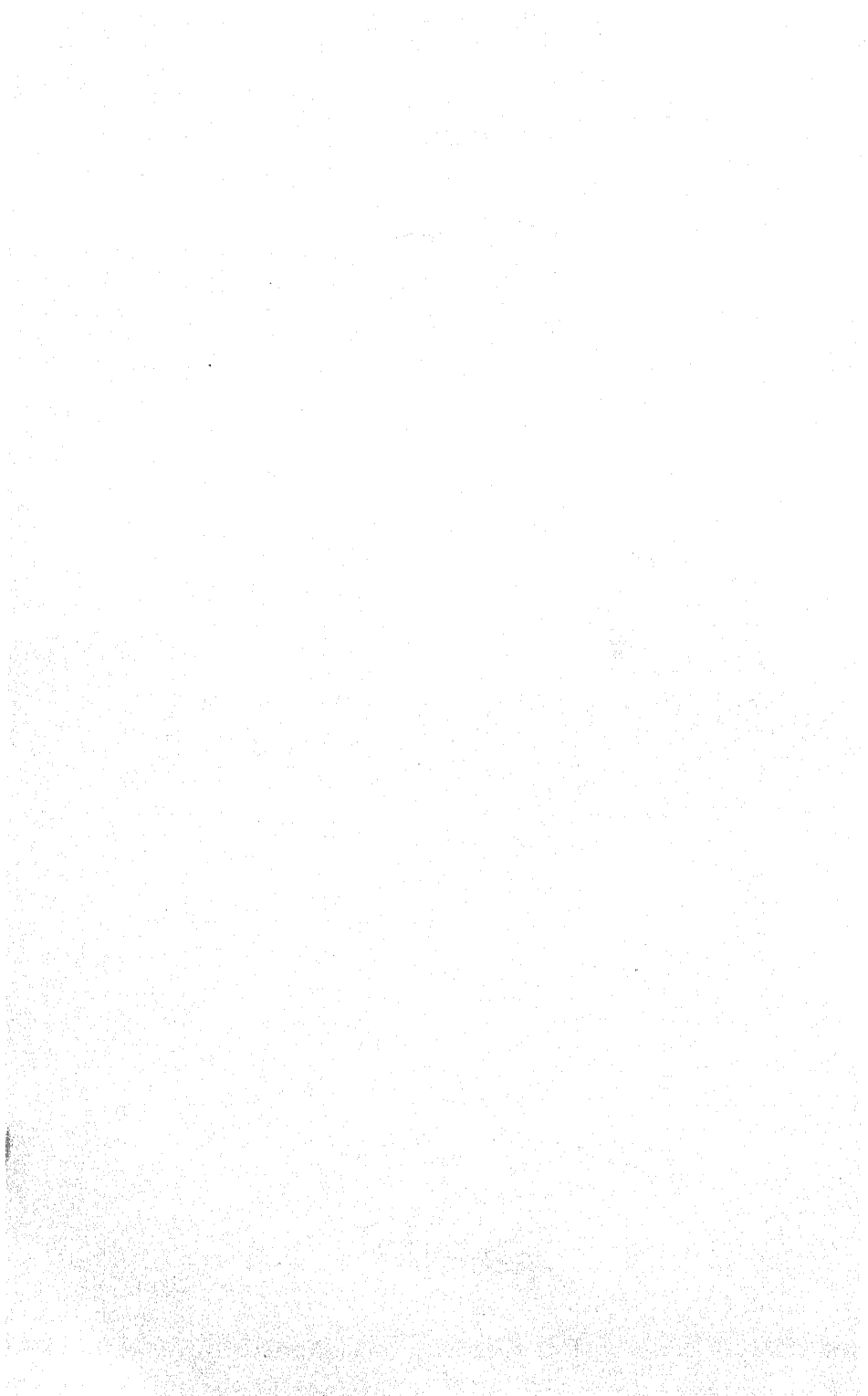
Pw

F1

Pb

ASHBY — INHERITANCE OF PHYSIOLOGICAL CHARACTERS.

Huth. London.



Studies in the Physiology of Parasitism.

XI. An Analysis of the Factors Underlying Specialization of Parasitism, with Special Reference to the Fungi *Botrytis Allii*, Munn, and *Monilia fructigena*, Pers.

BY

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With two Figures in the Text.

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A. INTRODUCTORY.

WHEN one thinks of the specialization of parasitism the examples which most readily come to mind are such groups of fungi as the rusts and mildews. With these the phenomenon is in many cases extremely sharply marked, as for example in the case of *Puccinia graminis*, strains of

which will only attack a limited number of varieties of a particular species of host plant. The explanation in physiological terms of this marked degree of specialization is practically non-existent. From microscopical observations certain facts are known, but it is only in such vague terms as 'Symbiotic relationship', 'Hypersensitiveness', &c., that the physiological relationships are expressed.

The obstacles in the way of physiological analysis in such cases are very great, and at the present time there is no immediate prospect of overcoming them. In the first place the high degree of specialization shown by such a fungus as a rust is associated with a highly selective metabolism, so much so that none of these fungi can be cultivated on artificial media. The only medium on which they will grow is the particular natural host. From the physiological point of view this is a medium of extreme complexity. Even if a complete chemical analysis of the host plant were forthcoming, it is not certain that one would be able thereby to prepare an artificial medium suitable for cultivation of such a fungus. A decoction of the host plant is no more suitable for the growth of these fungi than is any other medium.

Again, even if one could grow specialized fungi like rusts on artificial media of known composition, the study of their specialization would still entail great difficulties. The biochemical differences underlying their specialization are probably of an extremely subtle type. It has been suggested, for example, that specialization may be associated with the presence in different varieties of the host of different isomers of a particular plant product, e.g. carbohydrate or protein. Needless to say, the problem of investigating such minute differences is, with existing chemical methods, extremely difficult, if not impossible.

As contrasted with the specialized parasite, there is the type of *Botrytis cinerea* which is able to attack a considerable range of host plants, i. e., it represents the non-specialized type so-called. Associated with this feature of non-specialization is its capacity to be cultivated on artificial media. One is thus able to study the metabolism of such a fungus under comparatively simple conditions. Furthermore, its mode of parasitic attack renders it capable of biochemical study, at least to a certain extent. This fungus kills the cells of the host plant in advance of its growth. It is obvious, therefore, that it excretes an active principle into the cells of the host. The mechanism of the parasitic attack in this case is open to biochemical study, and the main outlines are fairly well known.

While a fungus of the latter type is comparatively non-specialized, there are nevertheless limits to its range of parasitic attack. There are many plants which *Botrytis cinerea* will not attack, even under the most favourable conditions; in other words, this fungus and fungi of similar type show some degree of specialization. Such specialization would obviously be described as of a cruder or coarser type than that seen in the case of

Puccinia, and one would anticipate that it would have its basis in correspondingly large chemical differences. The biochemical analysis of this type of specialization would offer some possibility of success, and the information so obtained might point the way to a better understanding of the more difficult problems of the highly specialized type of fungus.

The two fungi chiefly used in this investigation present a two-fold problem. *B. Allii* parasitizes onion tissue but, except to the limited extent which will be indicated later, is unable to attack apple fruit. The converse case is presented by *Monilia fructigena* which strongly attacks the apple, but under no conditions is able to produce the least attack on onion. The first problem, therefore, was to explain why *M. fructigena* failed to attack onion. As will be pointed out shortly, a satisfactory answer to this question was easily obtained. With the converse problem, however, of the failure of *B. Allii* to attack apple, much greater difficulties were encountered. The problem so presented has not been entirely cleared up, but a considerable amount of information bearing more or less directly on it has been accumulated. The study of the interrelations between *B. Allii* and the apple constituted much the greater part of the experimental work, and the record of these experiments represents the major portion of the present paper.

The particular problems outlined above have the advantage that the host parts are more or less massive structures. The experimental treatment, in so far as it involves surface sterilization, preparation of extracts, &c., is thus facilitated.

B. MATERIAL AND METHODS.

The three fungi used in this investigation were :

1. *Botrytis Allii*, Munn. Culture obtained from the Lister Institute, London.

2. *Monilia fructigena*, Pers. Stock culture kept in the Plant Pathological Laboratory, Imperial College of Science and Technology, London.

3. *Botrytis cinerea*, Pers. Stock culture kept in the Plant Pathological Laboratory, Imperial College of Science and Technology, London.

At the beginning of the work the purity of the various fungi was assured by taking a single hyphal tip in each case, after the method of Brown (4). Stock cultures were maintained in tubes of potato extract agar. The spores required for inoculation purposes were taken from cultures 10 to 20 days old.

Newtown apples were chiefly used, as these were available for most of the year. Other varieties tested from time to time were Winesap, Jonathan, Spitzenberg, and Bramley's Seedling. As regards onions, English or Spanish sorts were used, both giving similar results in this connexion.

Inoculation. In order to eliminate the effect of the cuticle as an

obstacle to infection, the fungal spores were introduced through wounds. In the case of inoculations on apples, the amount of attack was determined quantitatively, hence it was necessary to inoculate in as uniform manner as possible. After preliminary trials with needle and hypodermic inoculations it was decided that the most satisfactory method was that of Granger and Horne (8). This method was further standardized by the device of fixing a hard rubber ring to the cork borer used, so that the depth of the wound cavity was the same in all cases. In making an inoculation, a plug of tissue was removed, the spores inserted, and the plug replaced. The cut edges were then sealed up with paraffin wax. An uninoculated control was generally made on the same apple, the plug being merely cut out, reinserted, and the cut edges sealed up as before.

Infection by extraneous organisms was reduced to a minimum by washing the apples beforehand with absolute alcohol and wrapping them in grease-proof paper immediately after inoculation. The grease-proof paper had been previously sterilized by exposure for several hours to chloroform vapour. This method proved to be superior to dry heat sterilization, which renders the paper brittle.

At a suitable interval from inoculation each apple was weighed both before and after removal of the rotted portion. The amount of attack was given by the weight of rotted tissue. As at least two inoculations were generally placed on the same apple, it was important to interrupt the experiment before the two rotted portions had met. Any apple in which this had taken place was discarded. Where contamination was suspected several re-isolations were made, and unless the fungus originally inoculated was recovered free from admixture, the apple was rejected.

The following media, with or without agar, were used :

1. Glucose peptone :

Glucose.	10 gm.
Peptone.	2 „
Potassium dihydrogen phosphate.	1 „
Magnesium sulphate.	0.5 „
Distilled water.	1,000 c.c.

2. Brown's synthetic solution.

3. Potato extract :

Peeled potatoes	20 gm.
Distilled water to make up	1,000 c.c.

4. Onion extract

5. Apple extract

6. Turnip extract

} of full strength and in various dilutions.

C. EXPERIMENTAL.

This falls naturally into two sections :

1. The comparative study of *M. fructigena* and *B. Allii* in relation to onion.
2. The comparative study of *B. Allii* and *M. fructigena* in relation to apple.

(1) *Comparative Study of Monilia fructigena and Botrytis Allii in Relation to Onion.*

Extensive inoculations were made on onion scales and whole bulbs with the two fungi in question. For comparative purposes tests with *B. cinerea* were also included. Inoculations were made in a variety of ways. Spores were sown in water on the intact surface of the scales, or on the scales from which a portion of the epidermis had been stripped off, or in definite prick wounds. A number of whole bulbs were also inoculated by the plug method of Granger and Horne. Controls were made in the usual way by replacing the drops of spore suspension by drops of sterile water only. The inoculated scales were then stored in moist Petri dishes at laboratory temperature. The inoculated bulbs were similarly placed, or left in the open air of the laboratory.

The results obtained were uniformly as follows :

Both species of *Botrytis* attacked under all the experimental conditions tried. When the spores were sown on the intact epidermis attack was delayed by one or two days, but the final result was the same in all cases. Fungal hyphae were to be found in abundance in the rotted tissue.

With *M. fructigena* no attack took place under any circumstances. When the spores which had been placed in the onion tissue were subsequently examined they were found to be ungerminated.

As all the fungi are of the type which kill in advance of their growth, so that when parasitizing they grow in the escaped sap of the dead cells, the obvious point to examine was their behaviour towards onion extract. For this purpose, the juice of onions was squeezed out in a hand press and centrifuged in order to clear off the cell debris. Concentrated suspensions of the fungal spores were set up in distilled water, and one drop of these suspensions added to 2 c.c. portions of the onion extract. This process involves dilution of the latter by about 3 per cent. only, so that the final suspension is practically in onion extract of full strength.

In the comparative tests it was arranged that approximately the same number of spores per c.c. were present in each suspension. Sowings were made on thoroughly clean slides which were then kept in moist Petri dishes at 20° C. Similar preparations in which the spores were suspended

in sterile distilled water were used as controls. All the operations were carried out in as aseptic a manner as possible.

The state of germination was examined after 20 and again after 40 hours. It was determined quantitatively in two ways, (a) by counting the percentage of germinated spores, and (b) by measuring the length of 50 germ-tubes selected at random and taking the average.

Various tests were carried out in which onion extract, raw or heated or diluted with water, was used. Data illustrative of the results are given in Tables I and II.

TABLE I.

Fungus.		Raw Onion Extract.					Water.
		100 %.	50 %.	25 %.	10 %.	1 %.	
<i>B. Allii</i>	% Germination	0	72	99	100	98	96
	Germ tube (μ)	0	22	49	155	44	44
<i>B. cinerea</i>	% Germination	0	0	0	14	94	96
	Germ tube (μ)	0	0	0	70	92	95
<i>M. fructigena</i>	% Germination	0	0	0	0	86	74
"	Germ tube (μ)	0	0	0	0	v. long.	479

Duration of Experiment, 20 hrs. at 20° C.

TABLE II.

Fungus.		Onion Extract heated to 100° for five minutes.				Water.
		100 %.	50 %.	25 %.	10 %.	
<i>B. Allii</i>	% Germination	88	98	100	100	98
	Germ tube (μ)	24	22	92	275	44
<i>B. cinerea</i>	% Germination	99	100	100	100	94
	Germ tube (μ)	85	169	404	v. long	92
<i>M. fructigena</i>	% Germination	0	0	78	100	80
"	Germ tube (μ)	0	0	106	v. long	465

Duration of Experiment, 20 hrs. at 20° C.

In a further experiment onion extract was boiled for 20 minutes. This allowed free germination of spores even of *M. fructigena*.

The general results indicated may be summarized as follows:

1. The heated media allow more rapid germination in all cases, so that apparently the crude extract contains a substance which retards germination and which is driven off or de-activated by heat.

2. *Botrytis Allii* shows the greatest capacity to germinate in crude onion extract, and *M. fructigena* the least. *B. cinerea* is intermediate. With *Monilia* spores, the markedly inhibiting action of crude onion extract, even when diluted, is very striking.

After 40 hours, the only fungus which had germinated in the crude extract of full strength was *B. Allii*. The amount of germination was 100 per cent. *B. cinerea* failed to germinate after 80 hours, even in crude

extract which had been diluted to half strength. In the same time *M. fructigena* failed to germinate in the extract diluted to 10 per cent.

That the volatile principle of crude onion-extract plays a large part in the inhibitory effects above described is easily demonstrated by carrying out germination tests in which the filter-papers in the lids of the Petri dishes are wetted with crude extract. Under these conditions the amount of germination obtained is much less than when the filter-papers are moistened with water only.

The retarding principle of crude onion extract can be removed by extraction with chloroform, as in the following experiment.

A sample of crude onion extract was divided into two portions, one of which was shaken up with twice its bulk of chloroform in a separating funnel, the other meanwhile simply being kept in a corked vessel. After standing overnight, the chloroform layer was removed. The watery layer was then exposed for two hours in an open beaker. Germination tests were made in the untreated extract, in the water-soluble fraction, and in water. The results are given in Table III.

TABLE III.

Fungus.		Crude Extract.	Water- Soluble Fraction.	Water.
4 <i>B. Allii</i>	% Germination	0	40	98
	Germ tube (μ)	0	42	48
<i>B. cinerea</i>	% Germination	0	98	94
	Germ tube (μ)	0	471	90
<i>M. fructigena</i>	% Germination	0	66	68
"	Germ tube (μ)	0	238	469

In a control test it was shown that when onion extract was shaken up in chloroform, and the latter not separated off but simply allowed to evaporate, the inhibitory substance was still present in large degree. It is thus clear that the action of the chloroform is simply that of a solvent. Exactly similar results were obtained by the use of ether.

The retarding effect of onion extract on the growth of *Monilia* spores is further illustrated by the fact that an atmosphere containing the volatile principle markedly reduces the rate of attack of apples by that fungus. The following experiment illustrates this point.

Twenty-four apples were inoculated with spores of *Monilia*. Half of these were kept in a desiccator which contained 500 grm. of cut onion scales, the others in a desiccator without the onions. After five days the twelve apples kept over onions gave the following amounts of rotted tissue: 0.3, 0.6, 0.4, 0.5, 0.0, 0.7, 0.0, 0.4, 0.5, 0.4, 0.2, 0.7. Av. = 0.39 grm. In the corresponding set kept in the absence of onions, the amounts of rotted tissue were: 5.8, 5.7, 3.3, 3.1, 11.3, 5.0, 5.6, 4.0, 5.1, 3.5, 3.2, 2.4. Av. = 4.8 grm.

It is thus clear that the inability of *Monilia* to attack onion requires no further explanation beyond the fact that onion extract is markedly inhibitory to the growth of that fungus. It is noteworthy that even *B. Allii*, a parasite of the onion, germinates and grows better in boiled onion extract than in crude, so that it is likewise retarded by the volatile constituents of the extract. The ability of *B. Allii* to parasitize onion would thus seem to be correlated with its much greater tolerance of the volatile principle.

The failure of spores of *B. Allii* to germinate in crude onion extract for some considerable time may appear remarkable in view of its natural occurrence. In the experimental test, however, it is probable that the spores were subjected to a higher concentration of the active substance than would be the case in actual attack. As the fungus progresses, the volatile constituent is probably being continually dissipated, and thus it is readily understandable that spores that would not germinate under the conditions to which Table I refers would be able to grow under the conditions of parasitic invasion. Such an explanation is all the more necessary for *B. cinerea*, which is able to parasitize onion scales, but which is even more sensitive than *B. Allii* to the volatile principle.

The above described retarding effects of onion extract on fungal growth agree with similar observations made by Brown (3) and by Walker, Lindgren, and Bachmann (11). The latter worked with a large number of fungi, so that the results appear to have considerable generality of application.

(2) *Comparative Study of B. Allii and M. fructigena in Relation to Apple.*

(a) *Preliminary observations and experiments.*

Whole apples and apple-slices were inoculated from time to time with the fungi *B. Allii*, *M. fructigena*, and *B. cinerea* by all the methods mentioned above, but chiefly by the plug method. The spores were put on in the form of water suspensions. In the course of the work many hundreds of inoculations have been made with spores of *M. fructigena* and *B. cinerea*, invariably with success. On the other hand the 800 and more inoculations made with spore suspensions in water of *B. Allii* have given very little infection. The great majority gave none at all. In some cases when the inoculation method of Granger and Horne was used, some attack of the plugs took place, but subsequent work indicated that the latter result was probably due to the cork borer being rather hot at the time of cutting out the plug.

It was found that while the attack by *M. fructigena* varied considerably at different temperatures, *B. Allii* produced no attack at any temperature over the range 5°–40° C. The summarized data for *Monilia* are given in Table IV.

TABLE IV.

Temperature.	No. of Apples Inoculated.	Average Rot produced (grm.).
5° C.	8	0.63 ± 0.04
15° C.	18	17.4 ± 1.4
20° C.	17	33.2 ± 2.3
25° C.	18	37.5 ± 2.1
30° C.	18	49.5 ± 2.3
35° C.	18	No attack

Though no attack was produced with *B. Allii*, nevertheless microscopic and cultural observation showed that the spores were well germinated and capable of further growth. Nevertheless there still remained the possibility that germination took place so slowly that the apple tissue was able to set up some kind of resistance in the meanwhile. An experiment was therefore carried out to test the parasitic power of inocula consisting of *germinated* spores. The latter were prepared by sowing spores in sterilized apple juice. After two days, by which time a web of germ tubes had been produced, masses of germinated spores were placed in wounds on apple fruit. In this case again no attack resulted.✓

Quite apart from the preceding observation, it was found by actual test that the spores of *B. Allii*, *B. cinerea*, and *M. fructigena* all germinated with equal readiness in an apple extract of full strength, and that there was no definite difference between the growth obtained on boiled or unboiled extract. Table V gives the results of such an experiment.

TABLE V.

Fungus.	Germination Time.	Apple Extract.		Water.	
		Unboiled.	Boiled.		
<i>B. Allii</i>	12 hrs.	% Germination	86	81	64
		Germ tube (μ)	19	19	23
"	20 hrs.	% Germination	99	99	91
		Germ tube (μ)	126	112	40
<i>B. cinerea</i>	12 hrs.	% Germination	92	90	81
		Germ tube (μ)	112	77	81
"	20 hrs.	% Germination	100	100	95
		Germ tube (μ)	399	343	100
<i>M. fructigena</i>	12 hrs.	% Germination	71	55	45
		Germ tube (μ)	87	94	111
"	20 hrs.	% Germination	99	81	70
		Germ tube (μ)	259	181	483

The behaviour shown by *B. Allii* on apple tissue is thus different from that of *M. fructigena* on onion, and obviously therefore some other explanation of the failure to attack must be sought.

In a series of preliminary experiments, the method of inoculation was modified in a number of ways in order to eliminate certain factors which might be responsible for the failure of attack. These experiments will now be briefly described.

It was noticed that when a drop of water containing spores was placed in a wound on healthy apple tissue the drop disappeared after a few hours, even when the apple was kept in a saturated atmosphere. Apparently the water of the drop was absorbed by the neighbouring living cells. It was thought that scarcity of water might play a part in preventing attack. A number of apples were therefore inoculated and the drop of water renewed from time to time, other apples similarly inoculated but left undisturbed serving as controls. Even when thus plentifully supplied with water the spores of *B. Allii* failed to attack.

In the normal inoculations by Granger and Horne's method, the plug was inserted and the wounded surface of the apple covered with paraffin wax to exclude contamination. Under these conditions it was thought that accumulation of carbon dioxide or scarcity of oxygen might condition the failure to attack. These factors were, however, ruled out by the observation that no attack took place when the paraffin coating was omitted or when the plug was not inserted, so that the spores were directly exposed to the atmosphere. In one experiment the inoculated apples were kept in an atmosphere deprived of carbon dioxide, but again the same negative result was obtained.

The failure of *B. Allii* to attack apple would be explained if it could be shown that the fungus, when grown in apple extract, produced large quantities of staling substances sufficient to inhibit its own growth after a few days. This theory was tested as follows.

Spores of the fungus were sown in sterile 100 c.c. flasks, each containing 30 c.c. of apple extract of full strength, and also in a thin layer of the medium spread over horizontal glass plates. The latter method was considered to give a closer approximation to the conditions of germination in the inoculum. From time to time the liquid in which the spores had been sown was examined by centrifuging off the fungal material and sowing a fresh lot of spores in it. The amount of germination in the somewhat staled liquid was then measured. Similar germination tests were made at the same time in the fresh medium in order to control the effect of varying temperature from day to day.

TABLE VI.

Age of Stale Culture.	Growth in Stale Culture.	Growth in Fresh Culture.
Days.	μ .	μ .
2	18	18
5	73	20
7	112	33
9	94	24
12	75	18
14	88	24
21	53	32

The same type of result was obtained with the liquid from flasks or plates. Table VI gives an illustrative set of data, the figures in the table being the average length (μ) of 50 germ tubes after 24 hours' germination.

The variations shown in column 3 of the above table are chiefly due to varying laboratory temperature. A comparison, however, of the figures in column 2 with the corresponding ones in column 3 shows that spores of *B. Allii* germinate more rapidly in a so-called stale than in a fresh medium, at least within the limits of time of the experiment.¹ It is clear, therefore, that *B. Allii* when grown in apple extract does not produce a quantity of staling substances which would interfere with its further growth and which would explain the failure to establish parasitism.

The failure of *B. Allii* to attack apple would be intelligible if this fungus were unable to secrete a pectinase enzyme capable of attacking the cell-walls of apple tissue. The following tests were made in this connexion.

Apples were killed by freezing in an ice chamber, and after thawing they were inoculated, some with *B. Allii* and others with *M. fructigena*. Both fungi invaded these apples, but the actual limit of spread was not obvious to the naked eye. As a result of freezing the mechanical coherence of the apple tissue was considerably impaired, and one could not therefore be certain that the invading fungus had caused much softening of the cell-walls. The degree of invasion was determined by microscopic examination of pieces of tissue taken at different distances from the point of inoculation.

A more convincing result was obtained with apples killed by exposure to chloroform or ether vapour. The apples were kept in an atmosphere of chloroform or ether for four hours, then transferred to another vessel plugged lightly with cotton-wool and left for a week. By this time nearly all the gas had disappeared. They were then inoculated in the usual way. Both *B. Allii* and *B. fructigena* produced attack, especially along the surface. The last traces of gas were very slow to disappear, and this probably explains why both fungi avoided the central parts of the apples. Apples killed by the gas method still maintained coherent flesh, and therefore the progress of invasion by *B. Allii* could be determined by the rotting of the parts invaded. Distinct rotting was observed, and therefore it is clear that *B. Allii* is able to produce an enzyme which can attack the cell-walls of apple.

It is of course possible that treatment with chloroform or ether produces some chemical effect on apple cell-wall such as makes the latter susceptible to the action of the fungal enzyme. There is, however, no suggestion of such an effect in the known chemistry of cell-wall substance. Provisionally, therefore, one may assume that *B. Allii* is able to secrete an enzyme

¹ The more rapid germination observed in the 'staled' liquid is probably to be ascribed to a reduction in the concentration of osmotically active substances.

capable of attacking the cell-walls of living apple. Confirmation of this view will appear later.

The position reached is therefore as follows :

B. Allii is unable (normally) to attack living apple tissue, even though

- i. its spores germinate freely when placed on a wounded apple surface, and also in apple extract in full strength ;
- ii. its spores, on germinating, do not produce any staling substances which would inhibit their further growth ;
- iii. it is able to secrete a suitable pectinase enzyme when grown on killed apple tissue.

(b) *Attack of Apples by B. Allii when certain substances are added to the inocula.*

It has been known since the time of De Bary (5) that some fungi only attack plant tissue when they have previously had access to a food supply. De Bary's observations refer to the case where the fungal attack begins on the unwounded surface, and are to be interpreted in the light of the more vigorous growth which saprophytic nourishment makes possible. In the present work the invariable practice has been to insert the fungal spores into a wound on the surface of the host tissue, so that a supply of food, viz. from the cut and injured cells along the wounded surface, is already available for the fungus. As has been pointed out above, apple juice gives free germination to the spores of *B. Allii*, so that one would consider that the condition of previous saprophytic nourishment is fully met in the manner of experiment adopted. In view, however, of the failure to attack, it was decided to test whether any other kind of nutrient would prove to be effective.

Various concentrations of cane sugar, over the range 0.1–1.0 molar, were first tested. Spores sown in water served as controls. In another set of controls, drops of the various cane-sugar solutions, but without spores, were placed in wounds. In no case was any attack or injury shown.

The absorption of the liquid of the inoculum has already been mentioned. While no attack took place when spores were sown in a drop of apple juice, it was interesting to determine whether attack would follow if apple juice were repeatedly added to the spores in the wound. A batch of apples was therefore inoculated at three points on each, under the following three conditions :

- i. with spores in water,
- ii. with spores in apple juice,
- iii. with spores in apple juice, the latter being renewed every second day.

Controls were set up in which the same treatment was given, except for presence of spores.

In i and ii the fungus failed to attack and the controls remained perfectly sound. In iii there was a slight attack, but the controls also showed very much the same degree of injury. The repeated addition of apple juice to apple tissue apparently caused deleterious effects.

Similar experiments were carried out in which the effect of other media was tested. The media tested were as follows: onion extract, turnip extract of full strength and various dilutions of the same, Brown's synthetic medium, and glucose peptone solution. The possibility of injurious effects arising from the medium itself was controlled in the usual way. Table VII gives the average amounts of attack produced under the different experimental conditions.

TABLE VII.

No. of Apples.	Nutrient.	Average attack produced (grm.).
12	Onion extract	1.68 ± 0.19
8	Turnip extract (full strength)	1.08 ± 0.067
4	T. E., 50 %	0.72 ± 0.053
4	T. E., 20 %	0.43 ± 0.007
4	T. E., 10 %	0.15 ± 0.002
8	T. E., 1 %	0.12 ± 0.002
12	Brown's medium	0.63 ± 0.028
10	Gluc. peptone	0.34 ± 0.027
36	Water	No attack

It was thus clear that, in contrast to apple extract, all the above media gave definite amounts of attack. A series of experiments was therefore undertaken to determine whether such production of attack could be associated with any particular constituent of the nutrient medium. For purposes of this analysis Brown's synthetic medium was used. This has the following composition:

Glucose	2	grm.
Asparagin	2	"
K ₃ PO ₄	1.25	"
MgSO ₄	0.75	"
Water	1	litre

Inoculations were carried out in which the effect of the full medium was compared with that of a medium lacking in each constituent in turn. In order to eliminate as far as possible the factor of variable resistance in apple fruit, the inoculations were made in pairs on each apple. The results are given in Table VIII. The figures (grm. of rotted tissue) represent the average of 30 inoculations in each case.

TABLE VIII.

Medium.	Average Attack.	Probability.
Complete . . .	0·23 }	5 : 1
Lacking glucose . . .	0·14 }	
Complete . . .	0·20 }	> 100 : 1
Lacking asparagin . . .	0·06 }	
Complete . . .	0·22 }	3 : 1
Lacking K_3PO_4 . . .	0·20 }	
Complete . . .	0·22 }	—
Lacking $MgSO_4$. . .	0·22 }	

The third column in the above table gives the probability that the difference in amount of attack observed is valid. The method of calculation is taken from Fisher (7, p. 106). The calculations show that asparagin is the only constituent the omission of which very markedly reduces the amount of attack. The omission of glucose also reduces the attack, but not by a significant amount. The other two constituents are quite unimportant.

The results of Table VIII were confirmed by a further series of tests in which the attack produced in presence of the complete nutrient was compared with that due to each single constituent. It was then found that when the spores were sown in a solution containing 0·2 per cent. asparagin only, the amount of attack was the same as that on a full nutrient, within the limits of experimental error. On the other hand each of the remaining constituents produced only negligible attack.

The effectiveness of the glucose-peptone-mineral salts medium was similarly analysed, when it was found that the important constituent was the peptone.

In order to test whether a variety of nitrogenous compounds showed the same effect, a large number of inoculations were made in which the action of the following substances was compared—potassium nitrate, ammonium nitrate, ammonium sulphate, ammonium chloride, peptone and asparagin. A 0·4 per cent. solution of the latter was used, and the concentrations of the others were adjusted so that they contained the same amount of nitrogen. The summarized data are given in Table IX.

TABLE IX.

No. of Inoculations.	Source of N.	Average Attack (gram.).
96	{ Asparagin	0·25 ± 0·016
	{ KNO_3	0·20 ± 0·023
	{ Peptone	0·20 ± 0·023
	{ None (= water control)	No attack
100	{ Asparagin	0·35 ± 0·04
	{ NH_4Cl	0·45 ± 0·045
	{ NH_4NO_3	0·45 ± 0·02
	{ None	No attack
92	{ Asparagin	0·45 ± 0·044
	{ NH_4Cl	0·34 ± 0·04
	{ $(NH_4)_2SO_4$	0·34 ± 0·03
	{ None	No attack

All the sources of nitrogen tried produced significant amounts of attack. There was no indication that any one was superior to another when present in equivalent proportions.

The conclusion was thus reached that any source of nitrogen, when present in a proportion equivalent to 0.4 per cent. asparagin, was effective in stimulating attack by the spores of *B. Allii*. Further detailed work was then taken up, using asparagin only, to determine the relationship between the amount of asparagin and the magnitude of the effect produced, and in particular to find the minimum concentration of the chemical at which the effect could be detected. The general correlation between amount of asparagin used and the amount of attack is shown in Table X.

TABLE X.

Expt.	No. of Inocs.	Conc. of Asp. %	Amount of Attack. gram.
i.	{	12	0.32
		"	0.62
		"	1.13
ii.	{	11	0.51
		"	1.21
		"	1.58
iii.	{	8	3.25
		"	

In determining the minimum concentration of asparagin necessary to produce attack, it soon became evident that the amount varied with the state of ripeness of the apples. The variety of apple used was Newtown, which is in season from about November to February. Tests carried out in November showed that the addition of 0.1 per cent. asparagin to the inocula was insufficient to produce attack. Similar tests carried out in April, when the end of the season was approaching, showed that even when only 0.0025 per cent. asparagin was added, up to 50 per cent. of the inoculated apples became attacked. On the other hand, a concentration of quarter of that amount was insufficient at that time to stimulate attack. Table XI gives the approximate amounts of asparagin required to produce definite amounts of attack (0.8–1.0 gram. etc.) within fourteen days at different periods of the year.

TABLE XI.

Month.	0.8–1.0 gram. % Asp.	0.3–0.5 gram. % Asp.	0.1–0.3 gram. % Asp.
November	3	1	0.2
March	0.4	0.1	0.05
April (beginning)	0.4	0.025	—
" (end)	0.1	0.01	0.0025–0.005

The above figures show how the resistance of the apples is diminishing

as the season advances. However, as control inoculations showed, no attack took place when the spores were sown in water alone.

The presence of a trace of a nitrogenous compound in the inoculum not merely causes a fungus like *B. Allii* to attack apple, but accentuates the attack of an active parasite like *Monilia fructigena*. This is illustrated in Table XII.

TABLE XII.

No. of Inoculations	Spores of <i>Monilia fructigena</i> sown in—	Average Attack. gram.	Probability.
30	{ Water { Asparagin, 0.4 %	{ 1.85 } { 5.84 }	> 100 : 1
30	{ Water { KNO ₃ (= 0.4 % Asp.)	{ 2.29 } { 4.64 }	"
30	{ Water { (NH ₄) ₂ SO ₄ (= 0.4 % Asp.)	{ 2.57 } { 6.15 }	"

On the other hand it was found that the difference between the amount of attack produced when the spores of *Monilia* were sown in water and when they were sown in glucose solution was negligible. This is in agreement with the results for *B. Allii*.

(c) *Analysis of the results of the preceding section.*

In attempting to analyse physiologically the effect of increased parasitism due to the presence of a nitrogenous compound, attention was paid to two features of growth, (i) the amount and nature of the growth itself, and (ii) the amount of pectinase enzyme formed.

The effect of various concentrations of asparagin on the growth of *B. Allii* was studied in Petri dish cultures. For the sake of comparison *M. fructigena* was also included. The media used were Brown's synthetic agar and apple agar of different strengths, with additions of asparagin ranging up to 4 per cent.

The effect of varied asparagin on growth rate is negligible. On apple extract without asparagin the growth of both fungi is sparse and with rather feeble sporulation. The addition of a low concentration (0.2—0.4 per cent.) of asparagin much increases the amount of growth and sporulation, and further additions make little difference. So far as could be seen, the feeble growth of *B. Allii* was quite comparable to that of *M. fructigena* in the same circumstances, and there was no differential behaviour to suggest why the former should be non-parasitic and the latter parasitic on apple.

The study of enzymic relationships is incomplete and up to the present has been confined to *B. Allii*.

The enzyme solutions were derived, either from the liquid in which the spores of the fungus had germinated, or from an extract of the washed

germinated spores. The technique of the preparation was exactly as described by Brown (2) for *B. cinerea*. For testing the activity of various extracts, the material used consisted of discs of potato or apple of uniform thickness. These were cut by a hand microtome. The end-point was reached when the potato discs had lost coherence and when the apple discs had fallen apart into a mush. The two kinds of preparations, those obtained from the liquid of germination and those from the germ tubes themselves, will be referred to as 'External' and 'Internal Enzymes' respectively.

Table XIII gives the times necessary for the decomposition of potato and apple discs in the case of the external and internal enzymes of *B. Allii*, when grown under standard conditions on a variety of media. The activities of the various preparations are inversely as the times required for decomposition of the discs.

TABLE XIII.

Medium.	Potato Discs.		Apple Discs.	
	External Enzyme.	Internal Enzyme.	External Enzyme.	Internal Enzyme.
Onion extract	70 min.	60 min.	100 min.	45 min.
Turnip "	155 "	75 "	120 "	60 "
Brown's solution	155 "	—	285 "	—
Apple extract	> 40 hrs.	> 24 hrs.	> 40 hrs.	> 24 hrs.
A.E. + 0.4 % Asp.	17 "	90 min.	19 "	60 min.

The noteworthy feature shown in the above table is that both preparations from the cultures on apple extract have very slight enzyme activity, if any. On the other hand, when asparagin is added to the apple juice, quite a strong internal enzyme and a somewhat weak but definite external enzyme can be demonstrated. These observations have been repeatedly confirmed. Furthermore quite comparable results are obtained when the asparagin (0.4 per cent.) is replaced by an equivalent amount of potassium nitrate or ammonium sulphate, as is shown in Table XIV.

TABLE XIV.

Medium.	Potato Discs.		Apple Discs.	
	External Enzyme.	Internal Enzyme.	External Enzyme.	Internal Enzyme.
Apple extract	No action ¹	No action	No action	No action
A.E. + Asp.	21 hrs.	135 min.	18 hrs.	75 min.
" + KNO ₃	18 "	135 "	18 "	75 "
" + (NH ₄) ₂ SO ₄	21 "	105 "	21 "	60 "

¹ After 24 hrs.

The weakness of the extracts from the cultures on apple extract might be ascribed to the rather poor growth of the germ tubes in that case. There is greater growth on apple extract reinforced with an additional

source of nitrogen and especially on onion extract, and it may be suggested that the greater enzymic activity runs parallel with more vigorous growth. This, however, is not true. The feeblest growth of all was on Brown's synthetic solution, so much so that in the experiment shown in Table XIII, the amount of germ-tube material was insufficient for the test of the internal enzyme. Nevertheless the external enzyme was quite strong. Thus it is clear that the lack of activity shown by the extracts derived from apple extract cannot be explained on the basis of the somewhat feeble growth obtained.

Further experiments were carried out to determine whether the inactivity of the enzymic preparations derived from cultures on apple extract was due to lack of enzyme, or to the presence of some factor in apple extract which inhibited the enzyme. For this purpose a large quantity of dried and ground germ tubes was collected from cultures of *B. Allii* on onion extract. A standard extract of this powder was then prepared and a series of dilutions made, the diluting liquids being water, apple extract, apple extract with 0.4 per cent. asparagin, and onion extract. The activities of these various preparations were then tested in the usual way with the results shown in Table XV.

TABLE XV.

Concentrn. of Standard Ext.	Diluting Liquid.			
	Water.	Apple Ext.	A.E. + Asp.	Onion Ext.
Full strength	35 min.	35 min.	35 min.	35 min.
3:4	35 "	35 "	35 "	50 "
2:4	50 "	35 "	35 "	155 "
1:4	65 "	65 "	50 "	28 hrs.

The above figures refer to potato discs as test material. Quite similar results were obtained with apple discs.

There is clearly no evidence that apple juice contains any constituent which inhibits or even retards the action of the enzyme. On the contrary it appears that onion juice has a strongly retarding action on the enzyme, so that the high activity of the external enzyme prepared from onion juice is manifested in spite of such retarding substances.¹

Samples of apple extract in which spores of *B. Allii* had grown for two days and for four months were also tested after the manner shown in Table XV. Both these liquids when used for diluting the standard enzyme extract were quite comparable to water or to fresh apple extract in their effect on enzymic activity. Thus it is seen that neither fresh nor stale

¹ The retarding substance or substances present in onion juice may be to a large extent used up by the growing fungus, and therefore cease to affect the activity of the excreted enzyme. This point was not investigated.

apple extract has any marked inhibitory effect on the pectinase enzyme of *B. Allii*, whence it follows that the inactivity of the preparation from cultures on apple extract is entirely due to lack of enzyme. In other words, *B. Allii* does not secrete a demonstrable amount of enzyme when grown in apple extract.

That the fungus *B. Allii* when grown in apple extract does not develop a pH concentration sufficient to inhibit its enzyme was proved by direct experiment. The action of such a factor is disproved in fact by the results summarized in the preceding paragraph.

The effect of pH on enzymic activity was tested by adding various concentrations of acid (H_2SO_4) or alkali (NaOH) to a standard extract, determining the H-ion concentration by Clarke's method, and then testing on potato and apple discs. Fig. 1 shows the relation between activity (activity = inverse of the time required for decomposition of potato discs) and initial pH. The standard extract had a pH value of 6.0, and it was found that additions of acid up to pH = 3.8 had no measurable effect on activity. With additions of alkali, however, activity rapidly fell. The exact point at which de-activation took place was difficult to determine as the pH of the slightly alkaline enzyme solutions changed on account of the escape of juice from the test discs. The following figures illustrate this point :

Initial pH.	Final pH.
7.0	6.4
7.2	6.5
8.8	6.6
9.2	7.5

The dotted curve in Fig. 1 shows the relation between enzyme activity and final pH. The true curve would thus lie somewhere intermediately. The accurate determination would require the use of buffer mixtures in setting up the different H-ion concentrations, and probably also of comparatively large quantities of the enzyme solutions.

The variation of pH of apple extract in which *B. Allii* has grown is very slight. This range is indicated in Text-fig. 1 by the symbol x—x. It is obvious, therefore, that the fungus when grown in apple juice does not develop an H-ion concentration which would interfere with the activity of any pectinase enzyme produced.

(d) *Attack of apples which have been artificially 'ripened'.*

It has been already stated that the susceptibility of apples increases as the season progresses, so that a smaller concentration of nitrogenous compound is sufficient to initiate the attack of *B. Allii*. Just at the end of the season, when the apples (Newtown) available were yellow and very ripe, it was found that a certain amount of attack took place when the spores were

sown in water only. The following experiments show that this stage of susceptibility can be reached by what may be described as a process of artificial ripening.

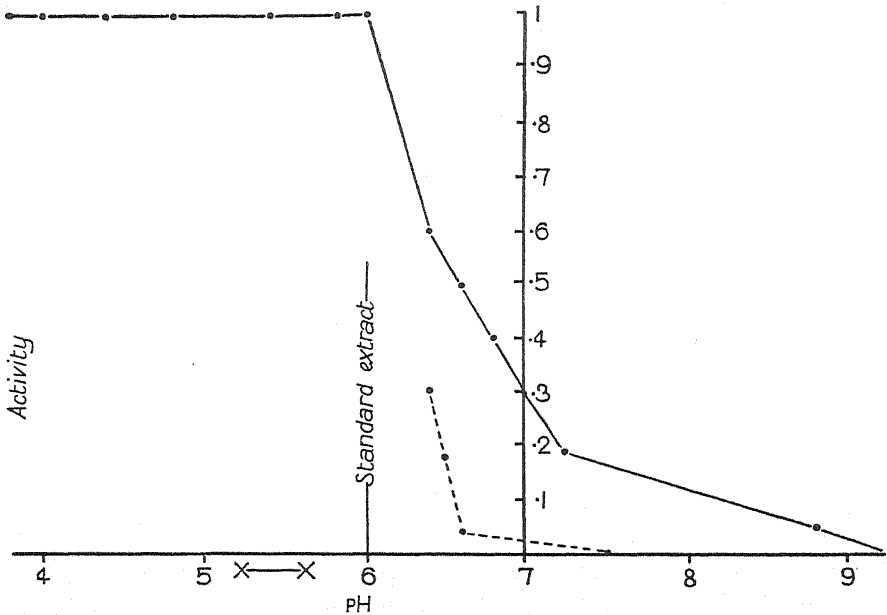


FIG. 1. Showing relation between enzymic activity and H-ion concentration.

The method is to keep the apples at a somewhat high temperature (30–35° C.) for some days, the length of time necessary depending on the temperature and the time of year. The record of a particular experiment is as follows:

Twenty apples were taken and divided into two lots. One of these was kept at 30° C. for seventeen days, the other at laboratory temperature for the same time. At the end of this period both lots were inoculated with a watery suspension of spores of *B. Allii* in the usual way. The heated apples showed the following amounts of rot (in grm.) after fourteen days: 2.5, 1.9, 2.7, 3.2, 1.6, 4.9, 1.8, 4.3, 3.5, 4.1, whereas the unheated were completely sound.

This result has been repeatedly confirmed.

Apples which have undergone the heat treatment are somewhat shrivelled from loss of water. The flesh when cut shows no discoloration, and when thin sections are placed in a hypertonic solution (20 per cent. KNO_3) they show plasmolysis. When a portion of skin is removed and the apple immersed in water it soon becomes firm. Thus there is no evidence that the treatment adopted has produced killing of the tissue.

The loss of water involved in the treatment is not responsible for the increased susceptibility. Heated apples are equally susceptible after they

have been placed in contact with water for a few days and thereby recovered from water loss. Similarly apples which are stored at laboratory temperature in a dry atmosphere (over calcium chloride) may lose the same percentage of weight, but are still immune to attack. It is known in fact that loss of water tends to increase the resistance of apples to attack, and therefore it follows that the heated apples are susceptible in spite of a somewhat low water content.

Analyses were carried out to determine whether the heating process had changed the composition of the apples in the three following respects :

- (i) Acidity of extract.
- (ii) Concentration of sugars in extract.
- (iii) Total nitrogen of extract.

The method of experiment was as follows :

Samples of about fifteen apples were incubated at 30°, and the same number kept at laboratory temperature. After 15–17 days all the apples were weighed and then halved. One half of each was inoculated in the usual way, the other half used for analysis. By this means it was determined whether the apples analysed were immune or susceptible. The inoculation tests invariably confirmed the results described above.

Determinations of acidity were carried out by the method employed by Archbold (1). The figures of Table XVI represent grams of malic acid present in 100 grams of apple juice, a correction being applied for the additional loss of weight which is incidental to the heating process.

TABLE XVI.

Expt.	Unheated Apples.	Heated Apples.
1.	0.406	0.218
2.	0.409	0.223
3.	0.441	0.228
4.	0.445	0.234
5.	0.523	0.312
6.	0.523	0.309

The heated apples have definitely a lower acidity than the unheated, but it is improbable that the difference in susceptibility to attack by *B. Allii* can be explained on this basis. Fig. 2, which gives the growth of *B. Allii* and *M. fructigena* over a range of acidity and alkalinity, shows that both fungi are very similar in their sensitiveness to acidity or alkalinity in the medium, and that both grow strongly in media more acid than the expressed sap of the unheated apples (pH, 5.2).¹

¹ The figures for pH given in the Table were determined on samples of the media which were autoclaved without addition of agar. They therefore do not correspond exactly to the actual pH of the solidified media but the error so introduced is slight.

Sugars. The sugars were estimated by the methods described by Evans (6) and by Lane and Eynon (10). Reducing sugars and total sugars were estimated separately, the difference being reckoned as sucrose. The data obtained are given in Table XVII.

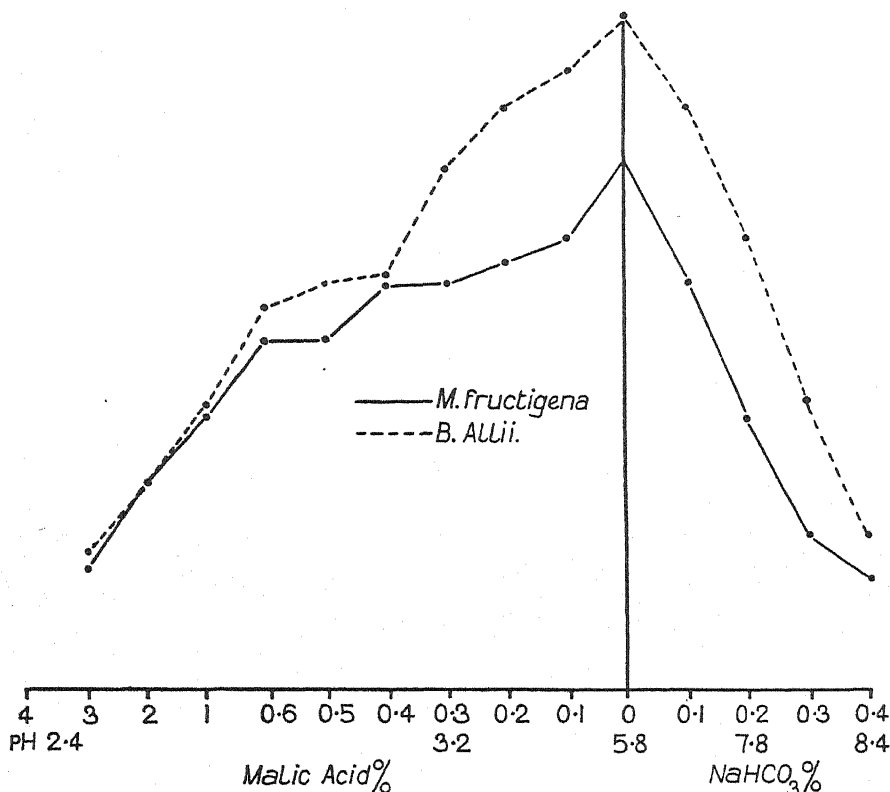


FIG. 2. Showing diameters of colonies (eight days' growth) of *Botrytis Allii* and *Monilia fructigena* in media of different pH (medium = glucose peptone).

TABLE XVII.

Expt.	Treatment of Apples.	Total Sugars.	Reducing Sugars.	Sucrose.
1.	{ Heated	11.64	11.23	0.41
	{ Unheated	11.57	10.74	0.83
2.	{ Heated	11.31	10.97	0.34
	{ Unheated	11.59	10.38	1.21
3.	{ Heated	12.69	10.69	2.00
	{ Unheated	12.83	9.18	3.65
4.	{ Heated	12.05	10.38	1.67
	{ Unheated	12.04	8.48	3.56

The heat treatment does not affect the total sugar content, but increases the reducing sugar at the expense of the sucrose. As it is, the small

changes in sugar concentration cannot be of any importance in explaining failure of attack in one case and success in the other. So far as is known, non-reducing sugar is as good as reducing for the growth of the fungus, and furthermore it was shown above that the addition of sugar to the inoculum had no definite effect on the amount of attack which took place.

Nitrogen. This was determined by the Kjeldahl method. As appears from Table XVIII no definite change in soluble nitrogen is produced as a result of the heating process.

TABLE XVIII.

Expt.	Nitrogen Content, (gm. N. in 100 gm. Apple Juice)	
	Heated.	Unheated.
1.	0.0165	0.0138
2.	0.0218	0.0115
3.	0.0226	0.0206
4.	0.0181	0.0209
5.	0.0230	0.0210

There is thus no evidence that the heated (susceptible) apples possess a higher content of extractable nitrogen than do the unheated (resistant).

At the moment, therefore, a biochemical explanation cannot be given of the change of resistance produced by the heating process. Some further points bearing on this question will be given in the following section.

D. DISCUSSION OF RESULTS.

The failure of *M. fructigena* to attack onion tissue is fully explained on the basis of the chemical properties of onion juice. The latter possesses a substance or substances which even in dilute concentration have a marked inhibitory action on *Monilia* spores. Attack is for this reason impossible. On the other hand, such a fungus as *B. Allii*, which parasitizes onion, shows a much greater tolerance of the active substances present in the tissue. The explanation given in this case has the merit of simplicity, but it is doubtful if it possesses any degree of generality. The parasite-host relationship is probably altogether a special one, arising from special properties of the host in question.

The converse problem of the failure of *B. Allii* to parasitize apple tissue offers much greater difficulties. Here the simple explanation given above does not suffice. The fungus freely germinates in contact with the tissue of the host and also in extracts of its sap, yet it does not parasitize. The main point brought out by the experimental work is that if a slight increase in the amount of nitrogen available to the fungus is given, definite attack takes place. Parallel enzymological studies show that no trace of a pectinase enzyme can be detected when *B. Allii* is grown in apple extract, whereas quite definite amounts are found when the apple extract is reinforced by

the addition of a trace of a nitrogenous compound. The resistance of apple tissue to attack by *B. Allii* is thus conditioned by its low nitrogen content. This observation is in agreement with the general view as to the role of nitrogen in relation to disease resistance, and in particular with the statement of Horne (9) that there is a definite correlation between the nitrogen content of different apples and the resistance of the latter to fungal invasion.

Nitrogen content, however, does not appear to be the sole factor which conditions resistance. Thus the heated and the unheated apples dealt with in the preceding section have much the same concentration of soluble nitrogen, and yet the former are susceptible and the latter not. The question therefore arises for further work as to whether there is some difference in permeability whereby the soluble nitrogen is able to diffuse into the inoculum in the one case to a greater extent than in the other.

A further factor, which the present investigation has not taken into account, is the resistance of the cell-wall. It is not improbable that the susceptibility of the latter to the action of fungal enzyme increases as the apple ripens, but this problem remains to be investigated. It is hoped that the method of ripening described in the preceding section will be useful in providing material for such an investigation.

In conclusion I wish to thank Professor W. Brown for suggesting the problem to me, and for his continuous interest and help throughout the course of the work.

E. SUMMARY.

1. An analysis has been carried out of the factors responsible for the failure of *M. fructigena* to attack onion and *B. Allii* to attack apple.

2. The chief feature shown by spores of *Monilia* when placed in wounds on onion is their failure to germinate. This is due to the presence of a thermolabile substance which can be extracted with chloroform or ether. This substance appears generally to retard fungal growth, but spores of *Monilia* are much more sensitive to it than spores of *B. Allii*.

3. Though spores of *B. Allii* are unable to attack apple tissue, this failure is not due to any inhibitory or retarding action of the apple juice on their germination, nor to any subsidiary factors such as lack of sufficient oxygen or over-concentration of carbon dioxide within the wound nor to self-staling of the fungus. There is also evidence that under suitable conditions *B. Allii* is able to produce demonstrable quantities of pectinase enzyme which attacks and destroys apple cell-wall substance.

4. *B. Allii* can be made to parasitize apple tissue by adding to the inoculum a certain concentration of a nitrogenous substance, such as asparagin, ammonium salt, potassium nitrate, or peptone. The addition of such substances also increases the rate of attack by *Monilia*.

5. The amount of nitrogenous substance which must be introduced to produce a definite amount of attack becomes less as the apples ripen, and finally a stage is reached at which attack occurs when spores are sown in water only.

6. The effect of a nitrogenous compound in stimulating attack by spores of *B. Allii* was found to run parallel with its effect in stimulating the secretion of the pectinase enzyme.

7. A method of artificially 'ripening' apples by exposure to somewhat high temperatures is described. By this process the apples are rendered susceptible to attack by *B. Allii*. The method will probably prove to be of value in providing material for the study of other factors (e.g. pectin relationships) concerned in disease resistance.

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A Note on the Dichotomous Branching of the Main Stem of the Tomato (*Lycopersicum esculentum*).

BY

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With three Figures in the Text.

FOR the purposes of the investigations on Virus Diseases carried out in this laboratory it is necessary to have a continuous supply of tomato plants. These are grown from seed and the seedlings are planted out in pots as required. It has been noticed that there is a tendency for the first foliage leaves to have an abnormal structure. One type of variation is not uncommon. The distal portion of the leaf is frequently forked so that the terminal pinnac are 'double' and the rachis is usually split so that there are two equal terminal pinnac. When this is so the two sides of the leaves are quite symmetrical. Among the plants under observation in the glass-house, however, appeared the present one in which the 'dichotomous' habit was much more evident.

The occasional appearance of forked leaves suggests that there is a tendency in the case of the tomato to have abnormal cell-divisions of the apical meristem which give rise to apparent dichotomy, and the occurrence of this plant indicates that this abnormality is not impossible of occurrence even in the stem apex.

The tomato plant has normally a three-fifths leaf-divergence and the branching is monopodial. In the plant under observation the first few leaves and the lower part of the stem presented a perfectly normal appearance. Almost immediately above the fourth leaf, however, the stem divided equally and gave the appearance of a dichotomously branching plant. There was no further dichotomous branching and the normal monopodial structure was continued. An examination of the two limbs of the plant showed that they were identical as regards both the number of the lateral members and the places of their insertion on the stem. It will be seen from Fig. 1 that each leaf on one branch has its fellow on the other.

Cases of simple dichotomy in Dicotyledons have from time to time been recorded. Worsdell (1) cites examples viz. the stems of the Jerusalem



FIG. 1. Tomato plant showing the bifurcating stem.

Artichoke (*Helianthus tuberosus*), of *Maesa ramentacea*, of Wall-flower (*Cheiranthus cheiri*) and the Stonecrop (*Sedum reflexum*).

In order to decide definitely that the present example was not merely an apparently equal development of the main stem and of an axillary shoot a study of the anatomy was made. Fig. 2 indicates the appearance of the stem at the place of forking. The leaf and its accompanying bud are clearly visible just below the region of division. This practically disposes of the possibility of one of the stems being axillary in origin. On the opposite side of each of the stems behind the rather swollen node are leaves each with an axillary bud, equal in size and corresponding in position. These represent the fifth leaf of the plant.

Further evidence that the stem was not axillary in origin was furnished by the examination of the vascular anatomy. In the tomato stem there are main groups of common bundles with connecting vascular tissue, forming a continuous stellar ring. When an axillary shoot grows out the vascular connexions are made entirely from the common bundle with which is connected the leaf in the axil of which it arises. It is thus a simple matter to recognize the out-growth of a lateral shoot from the examination of the vascular tissues of the stem.

In the present specimen there was a bud in the axil of the fourth leaf so that a few sections in this region sufficed to give examples of this type of vascular connexion. The vascular arrangement in the region of bifurcation is very different. The sections in Fig. 3 illustrate the salient features of the two types. The first section (3 *a*) was made just below the fourth leaf, the second (3 *b*) in the region of the fourth leaf, the third (3 *c*) between this leaf and the fork, and the fourth (3 *d*) at the lower end of the fork. Just above the fourth leaf the stem becomes rather flattened and increases in width in a direction at right angles to the length of the leaf. Thereafter a split appears in the vascular tissue, possibly associated with the difference in constitution of xylem tissue at this place

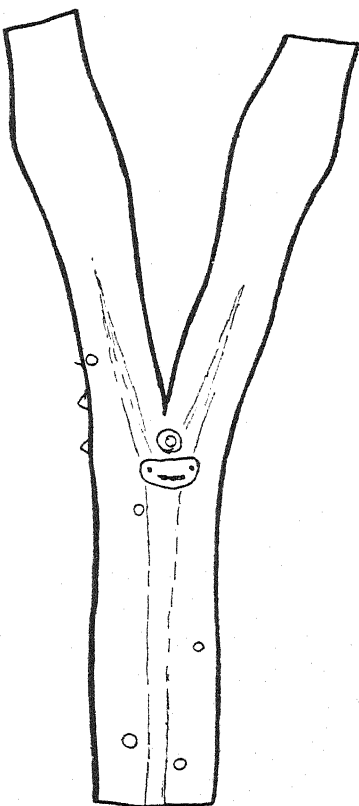


FIG. 2. Diagram of stem in region of forking.

and the initiation of the vascular tissues of two stems is evident. The point of interest is that in this case the two systems arise by the splitting of the stele of the stem. One is not formed, as in the case of an axillary shoot by the outgrowth of tissue from one of the common bundles of the main stem. Actually the division of two of the main vascular groups are to be seen in the sections. Each of the two limbs of the fork is therefore a true 'main stem'.

The evidence indicates, that in the present instance, the forking was due to a dichotomous division of the main stem and not to the over-growth of a lateral shoot with simulated dichotomy. The fact that the two stems are identical may be fortuitous or it may indicate that, the meristem having divided equally at an early stage, the resultant halves developed in exactly the same way. The latter seems the more probable explanation. The

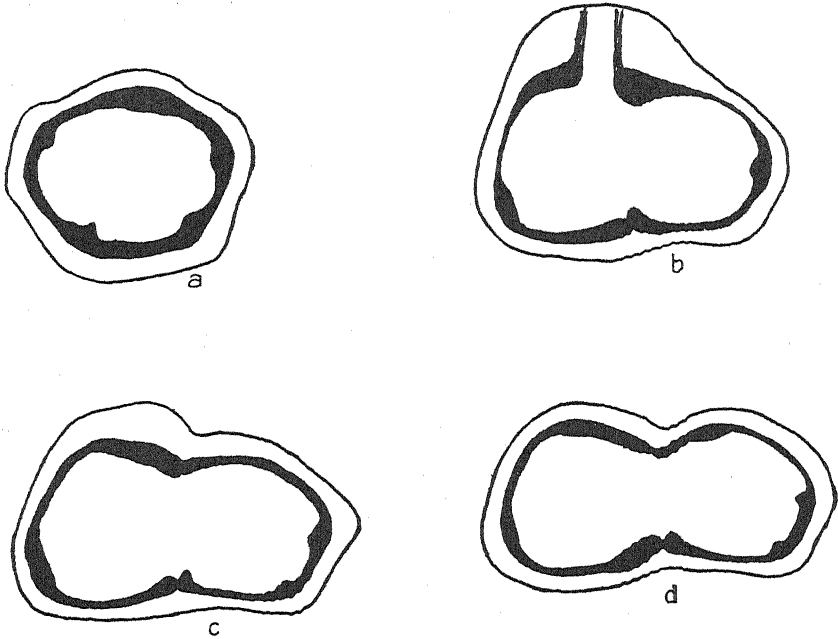


FIG. 3. Diagram of transverse section of stem at different levels. (a) 'Normal' main stem; (b) in region of fourth leaf; (c) between fourth leaf and fork; (d) at base of fork.

development of each of the parts seems to have followed the same course as would have been followed by the main stem had it continued normal growth under similar circumstances.

LITERATURE CITED.

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NOTE.

A NEW KIND OF POTOMETER.—Various forms of potometer are in current use by means of which the rate of transpiration of a shoot is deduced from the rate of movement of a column of water along a capillary tube. These are, on the whole, satisfactory instruments combining the advantages of simplicity, cheapness, and a reasonable degree of accuracy.

They give the average rate at which water is absorbed by the experimental shoot during the time taken by the column of water to move the measured distance along the capillary tube. It is not practicable to obtain satisfactory data as to variations in this rate within short periods, for a sufficient length of time must be allowed for the column of water to move a distance capable of accurate measurement.

The minimum time in which a reliable reading can be obtained will evidently depend on the vigour of transpiration—the more vigorous the transpiration, the more rapidly will the water column move a measurable distance. In other words, a longer and longer time must be spent on each observational period the smaller the shoot or the more sluggish the transpiration.

This relation holds with any form of potometer, but the limits beyond which it introduces serious practical difficulties is considerably extended in the potometer here described. This, while no less simple than the forms in common use, shows a much more rapid response to changes in the rate of absorption; hence, with the sort of shoot usually employed, a reliable measure of the rate of transpiration can be obtained in less than a minute, while an investigation as to the behaviour of a single leaf becomes practicable.

The form of the apparatus and the principle of its working are evident from Fig. 1, A.

The experimental shoot is fixed by means of a rubber stopper into one arm of a small U-tube. The U-tube is filled with water, and a glass tube is fitted into the other arm, also by means of a rubber stopper. This glass tube (the 'bubbling tube') is drawn out to a fine capillary at its lower end, the tip being bent round rather more than a right angle.

As the shoot transpires, it absorbs water from the U-tube; air, therefore, will be drawn through the bubbling tube and liberated from its end in the form of bubbles. By counting the rate at which the bubbles are produced a comparative measure of the rate of absorption of water by the shoot can be obtained.

If conditions remain constant, each bubble will be of the same size within a very small margin of error, and so the rate of bubbling will give an accurate measure of the rate at which water is being absorbed by the shoot. The fact that the temperature of the outside air may vary is of no importance, since the air drawn in from outside is within the bubbling tube for a sufficiently long time to acquire the same

temperature as the water in the U-tube, and the bubbles when liberated may be regarded as having this temperature.

A more serious error may be introduced by temperature changes in the pocket of air gradually formed by the accumulated bubbles at the top of the U-tube and, to a lesser extent, by temperature changes in the water itself. The importance of these

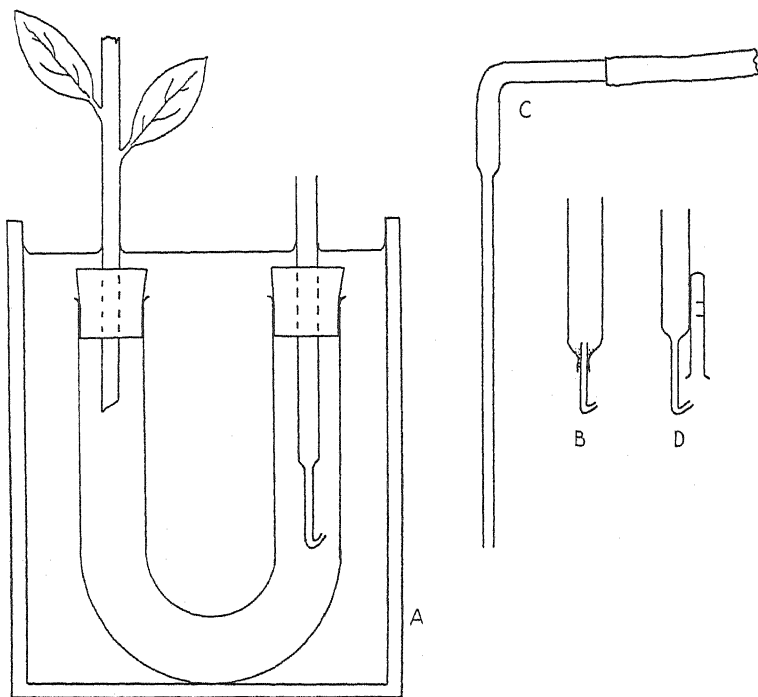


FIG. 1.

errors, however, can be for practical purposes eliminated by immersing the whole apparatus in a museum jar or beaker of water. Both the air and the water in the U-tube will then be maintained at the temperature of the surrounding water, and the large volume of the latter will preclude any sudden changes in its temperature. Moreover, slight temperature changes spread over a long period are of relatively small importance when comparison is being made between readings obtained at short intervals.

Needless to say, it is desirable to shield the jar from direct sunlight, and to take any obvious precautions against factors which might affect its temperature. Also, it is as well to leave the water which is to be used standing over night in a pail so that it may acquire the temperature of the laboratory, as water drawn directly from the tap is sometimes at a very different temperature; but this may be regarded as a desirable rather than as a necessary precaution.

The size of the air bubbles is controlled by the size of the capillary orifice, so that an apparatus with a fine capillary will be much more sensitive than one in which

the capillary tube is wider. If, however, the bubbles are too small, they do not become detached regularly from the bubbling tube, and in addition become difficult to observe. It must be remembered, also, that increased sensitiveness requires greater care regarding temperature variations.

For the most part, comparison of results yielded with the same instrument* is all that will be required. But if it be desired to have two instruments for comparison that will give off the same sized bubbles, two bubbling tubes with equal sized capillaries can be formed by drawing out a short length of glass tubing to form a capillary in the middle and breaking in two in this region. If several comparable instruments are required, a length of uniform bore capillary is broken into a number of half-inch lengths, and each of these sealed with sealing wax into glass tubes bent and narrowed somewhat at the ends (Fig. 1, B).

It is instructive to fit up an instrument in which a porous surface replaces the transpiring shoot in order that the relation between transpiration and simple evaporation may be determined.

It may be thought that the constant close attention required to record the exact moments when the bubbles are liberated, especially when the rate of bubbling is slow, is likely to be very wearisome. Observation is much less trying than might have been anticipated, however, for the following reason. The removal of water by the transpiring shoot gradually reduces the pressure within the U-tube until a bubble of air enters. This temporarily relieves the tension, and so water runs back some way into the capillary tube owing to surface tension. As the pressure within the U-tube gradually falls again, the water is slowly pulled along the capillary tube until this contains air only when, after a short pause, an air bubble issues from the end of the tube to be followed by another inrush of water. Concentrated attention is not required all the time, therefore, but only during the critical pause that occurs when the capillary tube is completely emptied of water. The position and behaviour of the little column of water in the end of the capillary tube provides adequate warning as to when the liberation of a bubble is likely to take place.

The last operation in setting up the apparatus is to insert the rubber stopper bearing the capillary bubbling tube into the second arm of the U-tube. One result of this operation is that water is forced into the bubbling tube for some distance. No bubbling will occur until all this water has been drawn back into the U-tube by the transpiring shoot. The following procedure is adopted to start the apparatus working and avoid waiting for the removal of the water in this way.

A piece of glass tubing is drawn out into a coarse capillary, narrow enough to be inserted into the open end of the bubbling tube and long enough to reach to the bottom of the wide part of this tube (Fig. 1, c). By sucking at the end of this, the water can be removed from all but the capillary portion of the bubbling tube. Withdrawing the bubbling tube a short distance through its stopper serves to remove the remainder of the water and fill the capillary part with air. The apparatus is now ready for use.

Generally it is sufficient to obtain comparative measurements of the rate of transpiration: if absolute measurements are required the bubbling tube of the apparatus must be calibrated. This may be accomplished by collecting the bubbles in a small measuring tube and ascertaining the number required to occupy a known

volume. A measuring tube for this purpose may be made as follows. A short length of glass tubing is sealed at one end. A small quantity of mercury is poured into the tube and the whole weighed, the height at which the mercury stands in the tube being marked on the glass. A small additional quantity of mercury is now added to the tube, the tube reweighed, and the new position of the mercury marked on the glass. From the two weighings the volume between the two marks can be calculated. The measuring tube, full of water, is now attached (e. g. with wax) to the bubbling tube with the open end downwards and above the capillary opening, so that any issuing bubbles enter the tube and collect at the closed upper end (Fig. 1, *d*). The number of bubbles is counted from the time the air column is level with the upper mark until it has reached the lower mark. With a measurer prepared in this way no error is introduced by the fact that the curve of the meniscus of the mercury used for calibration differs from that of the air-water surface.

(An account relating to a self-recording form of this apparatus will be published shortly.)

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The Structure of *Mesoxylon platypodium* and *Mesoxyloides*.¹

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With Plates XXV-XXVIII and one Figure in the Text.

MESOXYLON PLATYPODIUM, Scott and Maslen.

THIS is the only form of *Mesoxylon* of the five species named and diagnosed in our original note (6) which has not hitherto been described in detail.

As far as we are aware, only one specimen is known, and the delay in its description is partly owing to the hope that further specimens would come to light. This hope has not been realized, and the description is therefore based on the one specimen of a small stem, of which there are only two transverse sections and eight longitudinal ones.

The sections belong to the Scott Collection, now preserved at the Natural History Museum, South Kensington—Transverse sections Nos. 2384, 2385; Longitudinal sections Nos. 2386 to 2393 inclusive. This form, as were all the other species named in our note, was discovered by Mr. Lomax and his son in the Shore workings in the Lower Coal Measures of Lancashire, and the sections were cut by them.

As stated in our preliminary diagnosis (6, p. 239): 'This is the most isolated of the five species, and is characterized by the extreme separation (2 mm.) of the twin-bundles of the trace, and by their secondary division while still in the woody zone. The two axillary steles corresponding to a single leaf constitute a very striking feature. The specific name *platypodium* refers to the great breadth of the leaf-base.'

The specimen (Pl. XXV, Fig. 1) is small, broken, and much flattened. It measures about 3×1 cm., or probably about 2 cm. in its original round form. It is therefore smaller than most of the known specimens of other species of *Mesoxylon*.

At the stage in growth represented, the pith was relatively large, the woody zone and cortex together being only about 0.3 cm. in width. The

interior portion of the pith is not preserved, and it is therefore impossible to determine if the central pith was discoid, as in all other known species of *Mesoxylon*.

In other respects it exhibits the characters assigned to the genus: the persistent outer pith is followed by the zone of dense wood with, usually, uniseriate medullary rays, and that by the phloem, pericycle, and cortex, the latter strengthened by a system of sclerenchymatous bands of the *Sparganium* type; double leaf-traces with some centripetal xylem are present on the pith margin, and the leaf-traces undergo further subdivision in the pericycle and cortex before entering the leaf.

In *M. platypodium* the leaves were evidently widely spaced on the stem, as in a transverse section the outer surface shows only one slight protuberance (Pl. XXV, Fig. 1, *l.b.*) about 1.3 cm. in width, subtending a cortical leaf-trace of eight bundles—the only leaf-trace present in the pericycle or cortex. This protuberance evidently represents the position of the wide base of a leaf which became free at a higher level. The remainder of the outer surface is smooth and regular. Moreover, assuming that the stem is practically complete, though broken, only five clear leaf-traces are seen on the margin of the pith, and are thus widely separated from one another.

In the wide spacing of the leaves *M. platypodium* is in marked contrast with most of the species of the genus. In *M. Sutcliffii* the surface of the stem is practically covered with leaf-bases, indicating crowded leaves in a close spiral (1, p. 386). In *M. poroxyloides*, although the leaf-bases were probably less closely set than in *M. Sutcliffii*, there can have been no great extent of stem surface between them (3, p. 1022). In *M. multirame* the leaf-bases are also moderately crowded (4, p. 438). In *M. Lomaxii* the leaves appear to have been more widely spaced than in the three species mentioned (3, p. 1016), but, judging from the large number of traces around the pith, and from the fact that as many as four traces have been observed outside the wood in a transverse section, even in this species the leaves were much more crowded on the stem than in *M. platypodium*. How far such differences, in these fragmentary remains, are really of specific value, and how far they depend on differences in the order of the shoots and on nearness to the growing points, must remain for the present an open question.

The Pith.

The persistent outer pith shows but little differentiation. In transverse section (Pl. XXVII, Figs. 13 *o.p.*, and 14) it appears as a parenchyma of rounded cells with light brown contents, becoming somewhat smaller, and with darker contents towards the outside, especially opposite the outgoing traces. In the slight differentiation of the pith elements this species

appears to more closely resemble *M. Lomaxii* than the other species of the genus, in which there is a well-marked outer zone with dark contents abutting on the xylem ring.

In longitudinal sections the pith cells are seen to be very short, with an arrangement in vertical rows, the cells with darker contents forming a single layer next the xylem (Pl. XXV, Fig. 3, *o.p.* and Pl. XXVII, Fig. 16, *o.p.*). There is no clear evidence of elongated pith elements next the xylem, such as are seen in *M. Sutcliffii*, *M. poroxyloides*, and *M. multirame*.

As already mentioned, the inner pith is not preserved.

The Leaf-traces.

The leaf-traces exhibit the usual characters of the genus in being double where they leave the pith margin, and undergoing further division in the pericycle and cortex into eight bundles before entering the leaf. As in the other species, centripetal primary xylem is present. In this form the primary xylem of each bundle of the trace has divided into two, while the trace is still on the margin of the pith, evidently preparatory to the division of each bundle as a whole. This early subdivision of the primary bundles is a special feature of *M. platypodium*, as in all the other species the bundles show no sign of division until after their escape from the secondary xylem zone.

As already mentioned, another special feature is the wide separation, about 2 mm. (measured from the centres of the bundles), of the bundles of the incoming (or outgoing) trace, while still on the pith margin. In all other species of *Mesoxylon* the separation is much less. In *M. Sutcliffii* (l. p. 396) the corresponding distance is only about 0.9 mm., the bundles remaining separate for a long distance down. In *M. multirame* the traces on the margin of the pith show much the same characters as in *M. Sutcliffii*; the bundles are well separated and remain distinct for a long distance down, finally uniting into a single mass, but apparently without a definite fusion such as is seen in some other species. In *M. poroxyloides*, as the twin xylem strands come in through the secondary wood they converge, and almost immediately on reaching the border of the pith prepare to fuse, while at a slightly lower level the two centripetal arcs of xylem unite, so that the joint strand now has a single primary xylem mass. In *M. Lomaxii* a similar rapid convergence and fusion of the twin strands takes place.

Thus of all the species hitherto described the separation of the twin bundles on the margin of the pith is much greater in *M. platypodium* than in the others. In another form—*Mesoxylodes*—described in a later part of this paper, the separation is still greater, the tangential width of a leaf-trace being as great as 5 mm.

Some details of the leaf-trace in *M. platypodium* will now be given. The two transverse sections are fairly close together; No. 2385 is above

No. 2384. About six traces are visible, of which one consists of a long row of eight bundles in the pericycle (Pl. XXVII, Fig. 15), the others being cut at different levels in the wood. As seen on Pl. XXV, Fig. 1, the stem is broken, and may be incomplete, but it is unlikely that much is lost.

One of the leaf-traces at the edge of the pith is shown on Pl. XXVII, Fig. 13. The two strands are about 2 mm. apart (centre to centre); each shows an arc of centripetal xylem, with possibly a little non-radially arranged centrifugal primary xylem on the distal side of the small-celled thinner-walled elements marking the position of the protoxylem. But neither *M. platypodium* nor any of the other described species of the genus afford any definite evidence of the mesarch structure suggested in the description of *M. Sutcliffii* (1, p. 395), although in the allied genus, *Mesoxylopsis* (5, p. 15), mesarch structure is evident.

In each bundle the primary centripetal xylem is double; this is best shown in the one on the left in Pl. XXVII, Fig. 13. As already mentioned, this early division of the centripetal xylem is a diagnostic feature of this species. This trace is evidently on the point of passing out through the centrifugal woody zone, since in the section above (No. 2385) the twin bundles are seen just escaping from the wood, one, the left one in Fig. 13, farther out than the other. In this higher section the secondary wood has closed in behind the bundles, more completely in the one farther out, which now shows very clearly the two arcs of centripetal xylem subtending two distinctly separated spaces representing the protoxylems. The distance between the two bundles is only slightly greater than when on the pith margin. Although an early separation of the primary xylem of each bundle thus takes place, no division of each bundle as a whole takes place until it has escaped from the wood, agreeing in this respect with the other species.

Other traces on the pith margin show the characters when cut at lower levels. Several of the bundles still show clearly the separation of the primary xylem, so that the division must take place at a level considerably below that at which the trace passes out. But the bundle illustrated on Pl. XXVII, Fig. 14, one of a pair still separated about 2 mm., shows no clear indication of separation in the primary xylem, x , which forms one continuous mass. Another trace, with bundles still over 1.5 mm. apart, shows very little centripetal xylem, and it seems probable that it disappears altogether long before actual fusion of the twin bundles takes place.

The convergence downward of the two bundles of the trace must be very slow, as the distance apart of the two is about 2 mm., or only slightly less, in several traces which must pass out at different and considerably higher levels.

There is considerable difference in the species of *Mesoxylon* as regards the place or level of appearance (or disappearance, according to which way

the trace is followed) of the centripetal xylem. In *M. Lomaxii* and *M. poroxyloides*, in both of which the twin bundles fuse almost immediately on reaching the pith margin, the centripetal xylem (as a single mass) persisted downward for a considerable distance before it eventually died out. In *M. poroxyloides* it has been possible to demonstrate the actual way in which the two centripetal arcs of xylem unite to form the single primary mass of the joint strand (3, p. 1019). In *M. multirame* and *M. Sutcliffii*, on the other hand, in which the twin bundles remain distinct for a considerable vertical distance downward after reaching the pith margin, there is probably never a definite fusion, such as is seen in the other two species, the two bundles, together with the median strand, merely grouping themselves in one mass. But whereas in *M. Sutcliffii* the centripetal xylem persists downward to about the level at which complete coalescence of the twin bundles takes place, the previously distinct inner wood of the two bundles losing the arc form, and forming one straight band before it finally disappears, in *M. multirame* the centripetal xylem has already ceased to be distinguishable before the grouping of the bundles is accomplished, so that the joint bundle is definitely exarch in structure.

Finally, in *M. platypodium*, with its still wider separation and very slow convergence of its trace bundles, the centripetal xylem apparently disappears long before the approximation of the trace bundles takes place.

A feature often seen in this species is a certain degree of asymmetry between the two bundles as seen in transverse section. The separation of the primary xylem into two is often more clear in one bundle than in the other, as shown on Pl. XXVII, Fig. 13, where the left-hand bundle shows the separation more clearly than the other (the difference is rather more clear in the section than is shown in the drawing). In the section above (No. 2385) the same trace is seen passing out through the wood, which has closed in behind the bundles, but one of the bundles—that with the more complete separation when on the pith margin—is considerably farther out than the other.

In *M. platypodium* no bundle sheath is developed separating the xylem of the perimedullary strands from the pith, such as forms a characteristic feature in *M. Lomaxii* and *M. multirame* (3, p. 1014, and 4, p. 447).

Some of the longitudinal sections pass through the traces on the edge of the pith. As in other species of *Mesoxylon* the centrifugal xylem of the bundles presents a different appearance from that of the ordinary centrifugal xylem; the tracheides of the bundle follow a somewhat undulating course, with relatively broad medullary rays between them, the structure being quite different from that of the ordinary secondary wood with its closely packed straight tracheides and narrow medullary rays. The centrifugal xylem elements of the bundles appear to be mainly of scalariform or tightly spiral type, passing gradually outward, through intermediate reticulated elements,

into the pitted tracheides which form most of the secondary xylem zone of the stem. The centrifugal xylem of the traces is essentially like that shown in the figures of *M. Sutcliffii* (1, Pl. XXXIII, Fig. 6, and Pl. XXXV, Fig. 14).

The centripetal xylem and protoxylem are not well shown in any of the longitudinal sections, but in No. 2387, which is tangential through the wood and outer pith, possible spiral protoxylem is seen at one point, and in No. 2388 centripetal xylem is shown in two places. Again, in No. 2389, a radial section, at one place a lax spiral protoxylem element is seen next the pith, doubtless belonging to a strand cut low down in its course, after the disappearance of its centripetal wood.

Thus the structure of the bundles on the pith margin appears to be similar to that of the other species of the genus.

Following now the leaf-traces in their course outward from the pith margin, the pair of bundles shown on Pl. XXVII, Fig. 13, *l.t.*, *l.t.*, is seen in the higher section (No. 2385) to be passing out through the woody zone, the wood having closed in behind them. But, as already mentioned, one of the bundles is considerably farther out than the other, and this one now shows very clearly the two separate arcs of centripetal xylem and two spaces representing the protoxylems.

A similar asymmetry is often observed in *M. Sutcliffii*, in which one bundle may be well out in the phloem while the other is still embedded in the wood. As the bundles are cut almost transversely they must follow a steep course through the xylem ring. The almost parallel course of the bundles at the pith margin noted above is evidently maintained through the wood, as their distance apart is only slightly greater than when on the edge of the pith.

In section No. 2384 the trace shown in Pl. XXVII, Fig. 15, is seen. Here it consists of the usual eight collateral bundles in the pericycle, as in the other species. The eight bundles are distinctly in two sets of four each, the innermost and outermost bundle of each set being larger than the intermediate ones. The two innermost bundles are only slightly farther apart (about 2.5 mm., centre to centre) than when still on the pith margin, while the whole tangential width of the eight bundles is about 0.8 cm. No indication of asymmetry is visible at this level, i.e. the four bundles of one side of the trace, which have doubtless resulted from the division of one of the original ones, are symmetrical to those on the other. But, as seen in Fig. 15, the bundles are not equidistant from the outer edge of the xylem ring, the two more central ones being the nearest, and the distance gradually increasing to the end of each row.

In the higher section (No. 2385) the same trace is again seen somewhat farther out, near the junction of pericycle and cortex. Here the same eight bundles are seen, the distance between the inner ones practically the same

as before, with the same feature, as noted above, in the distance outward of the respective bundles.

In Pl. XXV, Fig. 2, *l.t.*, *l.t.*, *l.t.*, three of the eight bundles are shown, corresponding with three of the right-hand set seen in Pl. XXVII, Fig. 15. In this higher section the two end bundles, i.e. those farthest out, appear in oblique section (the others being cut approximately transversely), and show some indication of division, and are evidently bending out more horizontally and dividing as they prepare to enter the leaf. In this section a distinct protuberance has appeared, about 1.3 cm. wide (Pl. XXV, Fig. 1, *l.b.*), indicating the position of the leaf base. Corresponding to it there is a marked thickening of the pericycle, in the outer part of which the trace is still embedded. The bundles in the pericycle (Pl. XXV, Fig. 2, and Pl. XXVII, Fig. 15) have the usual structure, such as was described in *M. Sutcliffii* (1, pp. 402, 403) and other species, in which the preservation is better than in *M. platypodium*.

A marked feature of the bundles in this form is that they carry out with them an arc of the cells with very dark contents which form a definite zone outside the phloem of the main axis (Pl. XXV, Fig. 2, *s's.*, and Pl. XXVII, Fig. 15, *s's.*).

The longitudinal sections yield little detailed information respecting the leaf-traces outside the woody zone. Slide No. 2386, which is tangential through the cortex, shows oblique sections of a number of bundles, in which little can be made out excepting that the tracheides are mainly scalariform. Slide No. 2392 also shows bundles in the cortex, again cut obliquely and with scalariform or spiral tracheides.

The Axillary Steles.

A striking feature of *M. platypodium* is the presence of *two* axillary steles corresponding to a single leaf. Axillary steles have been described in two other species of *Mesoxylon* (*M. Sutcliffii* and *M. multirame*). In *M. Sutcliffii* (1, p. 407) twin bud steles are occasionally seen embedded in the pericycle or the inner cortex, but in general only a single stele is shown in the transverse sections, even when quite close to the secondary wood, which seems to indicate that the division of the bud stele, if it took place, occurred while it was traversing the zone of wood. In this species it was not possible to trace the single stele inward through the wood to see if, and how, this division took place. Moreover, in *M. Sutcliffii*, successively higher sections show that even when two steles were present they united at a higher level before entering the bud. In *M. multirame* (4, p. 448), which in many respects closely resembles *M. Sutcliffii*, the single axillary stele, as it passes through the cortex, has the same structure as in the latter species, although the nature of the shoots from which they come (or into which they

pass) is very different, the leafy buds of the one contrasting strongly with the naked elliptical shoots of the other, the one probably vegetative and the other reproductive. In the present connexion, however, the most interesting point is that in *M. multirame* the axillary stele often divides into two as it approaches the primary wood of the stem. This division was not, however, constant in this species, as many of the axillary steles persist as single strands right through to the pith edge. In *M. multirame* it was possible to obtain evidence as to the relation of the axillary stele to the primary bundles of the main axis, and to show that the stele in its vascular connexions follows the coniferous type, i.e. it is not directly connected with its own subtending trace, but with the trace bundles to the right and left, between which its own trace passes out. A similar connexion was found by Bertrand and Renault in *Poroxylon*.

In *M. platypodium* the two steles are not only widely separated when in the pericycle and cortex, but retain their separation right through the xylem ring to the pith margin, in correspondence with the wide separation of the bundles of the trace which they follow out.

In the lower of the transverse sections (No. 2384) there appear two gaps in the xylem ring, within the eight-bundled leaf-trace, as shown on Pl. XXVII, Fig. 15, *s.g.* These gaps are about 2.8 mm. apart, and doubtless correspond to the two axillary steles seen in the higher section at a slightly greater distance apart. No well-defined steles are actually visible in these gaps, but they are not as completely empty as would be inferred from the semi-diagrammatic drawing (Pl. XXVII, Fig. 15). Short radial rows of loosely arranged tracheides partly occupy these gaps, and the tracheides of the adjacent xylem ring on both sides are disturbed, those on the 'outer' side of each gap curving round at the inner ends of the radial rows towards the gaps, thus confirming, apparently, the mode of origin of the axillary steles in *M. multirame* mentioned above.

Fortunately, one of the longitudinal sections (No. 2391), intersects a pair of steles on the inner edge of the wood. This section cuts the wood and outer pith more or less tangentially; it also intersects two bands of tortuous interlacing tracheides. Part of one of these bands is shown on Pl. XXVII, Fig. 16, *t.* The two bands evidently represent the two steles passing vertically along the inner edge of the xylem ring.

Some of the tracheides are shown on a larger scale in Pl. XXVII, Fig. 17. The distance between the two bands is about the same as that between the two gaps in the transverse section (Pl. XXVII, Fig. 15, *s.g.*). Between the winding elements are variously orientated medullary ray cells, often single, sometimes in rows of two or three, and sometimes in small masses of a few irregularly-arranged elements. The structure is quite different from that of either the centrifugal or centripetal xylem of the leaf-traces. The bands are clearly on the pith edge, for, as shown in Fig. 16, pith cells, *o.p.*, are

seen quite close to them. The other band would be to the right in the figure.

The band figured can be traced upward through the slide for a distance of 1 cm., and this is not its full length, as it evidently extended beyond the margin of the section. Clearly the origin of the xylem of the steles is a long distance below the level at which they pass out. Following the stele shown in the figure downward for the distance mentioned above, we see what may be its place of origin. Here, in actual contact with the pith cells, are seen some small spiral elements. The winding tracheides of the bands show no scalariform or other markings. Traced downward, below the level at which the spiral elements are seen, and in line with the stele band, is a tissue consisting of the ordinary straight scalariform tracheides, apparently representing the bundle from the protoxylem of which the stele xylem takes its origin.

Between the two stele bands appears the tissue, of which part is represented at *i.x.* in Pl. XXVII, Fig. 16. This clearly represents the intermediate xylem seen between the gaps in the transverse section (Pl. XXVII, Fig. 15, *i.x.*). This tissue is perfectly continuous laterally with the tortuous bands of the steles on either side at the upper end of the section, and here it has the ordinary compact structure of the secondary xylem of the main axis, but traced downward, owing to slight obliquity of the section, it gradually wedges out, pith elements appearing on both sides (one side is shown in Pl. XXVII, Fig. 16, at *o.p.*), and the structure gradually gives place to the looser type of xylem characteristic of the centrifugal xylem of the leaf-traces, with spiral or scalariform thickening.

The reason for the wedging out, as the section approaches the pith, will be evident from the shape of the intermediate xylem, as seen in Pl. XXVII, Fig. 15.

In the transverse section (No. 2385), above that from which Pl. XXVII, Fig. 15, is drawn, the two steles have emerged from the xylem ring, and are seen in the pericycle within the leaf-trace. One of the steles, together with three of the eight leaf-trace bundles, is seen in Pl. XXV, Fig. 2, *a.s.*, the bundle on the left being one of the central ones, corresponding to Pl. XXVII, Fig. 15, *l.t.* The steles are now about 3 mm. apart, and exhibit a structure essentially similar to that of the steles in a similar position in *M. Sutcliffii* (1, Pl. XXXIV, Fig. 11) and in *M. multirame* (4, Pl. XIII, Fig. 21), with the same tangentially elongated form. The size of the one shown in the photograph is about 0.40 x 0.23 mm., to outer edge of xylem only, and is smaller than in either of the other species. As already mentioned, the stem of the only known specimen of *M. platypodium* is also smaller than in the other species.

The stele shown is cut almost transversely, indicating that, like the trace bundles, it is following an almost vertical course through the pericycle. Pl. XIII, Fig. 21, in the *M. multirame* paper, sufficiently illustrates its

general appearance and structure, excepting that in *M. platypodium* the number of xylem wedges is less and the centrifugal wood is only about four to six tracheides in thickness. Wide medullary rays, of which the cells are not preserved, separate the xylem wedges, as in the other species. The phloem zone surrounding the xylem is not preserved, but it appears to be very narrow. A pith is present, but no clear centripetal xylem at the inner ends of the wedges can be seen, probably owing to poor preservation.

The two steles are not quite symmetrically placed with reference to the trace; this may be due to displacement.

No other pair of steles is visible in either of the transverse sections, but a single stele is seen rather farther out in the pericycle in the lower slide (No. 2384), on the opposite side of the stem to the stelar gaps previously described. This stele is shown in Pl. XXVIII, Fig. 18. It is considerably longer in the tangential direction than the others, being 1 mm., which is more than double that of a stele farther in, while the diameter in the radial direction is about the same as before.

This stele is cut slightly obliquely, and has a somewhat different structure from that of the steles described above. There is a clear pith of relatively large rounded cells, *p.*, but the radial rows of centrifugal tracheides are absent in the central portion of the inner side, the tracheides appearing towards the ends of the elongated stele, and being fully developed in rows of about half a dozen elements on the outer side. Thus this stele appears to be 'open' on its axial side, its pith elements being there continuous with those of the pericycle, *pe.*

Unfortunately, this stele cannot be seen in the higher section.

It is suggested that this stele is not one of a pair, but represents a stage in the opening out and fusion of two smaller steles coming up from below, and before its entry into the bud or branch.

The rather thick-walled pith cells (Pl. XXVIII, Fig. 18, *p.*) also appear to be in two groups, separated by thinner-walled elements.

The longitudinal sections yield no information as regards the axillary steles in the tissues outside the xylem.

Thus, both in *M. platypodium* and in *M. Sutcliffii* (in which two steles are sometimes present in the pericycle or cortex) there is evidence that the two steles fuse into one as they pass upward and outward.

The Xylem, Phloem, Pericycle, and Cortex of the Main Axis.

Xylem. The thickness of the woody zone is about 1.2 mm., and it has the compact dense structure characteristic of *Mesoxylon* and of other Cordaitales, with radial rows of secondary tracheides, separated by narrow uniseriate medullary rays (Pl. XXVII, Figs. 13 and 16, *x.*). The tracheides are generally 30–40 μ in diameter, becoming smaller where they abut laterally on the bundles of a leaf-trace.

Radial sections (Nos. 2388, 2389, and Pl. XXV, Fig. 3, *p.t.*) show that the greater part of the centrifugal xylem consists of pitted tracheides which are followed towards the pith by scalariform elements. Transitional reticulated tracheides occur, and the first-formed pitted ones commonly have their pits in about three rows, while in those farther out the somewhat larger pits are frequently in two rows, or in only one. The width of the zone of scalariform tracheides adjacent to the pith varies considerably in different places; sometimes only one such element appears next the pith; at other places as many as eight are seen. The greater thickness is probably due to slight obliquity of the section, owing to which it passes through the centrifugal wood of the lower part of a leaf-trace after the centripetal xylem has disappeared. At one place in slide No. 2388 a pitted element abuts directly on the pith, but this is unusual; at another place a lax probably spiral element is seen next the pith, probably from a leaf-trace cut low down in its course.

The secondary medullary rays in the radial sections have the usual appearance (Pl. XXV, Fig. 3, *m.r.*), and vary in height from one to about fourteen elements. Large pits occur on the radial walls between the tracheides and the medullary ray cells.

Tangential sections of the secondary xylem (Pl. XXVII, Fig. 16, *x.*) show the usual tightly packed nearly parallel tracheides, separated by narrow rays. No pitting is visible on the tangential walls, such as was observed in *M. multirame* and *Pitys antiqua* (2, p. 352). Traced towards the pith the xylem becomes of a looser type, with more undulating scalariform tracheides and broader medullary rays, similar to that of the centrifugal part of the leaf-traces, and on which, of course, the thickened bars extend all round the tracheides. No xylem parenchyma has been observed.

The general structure of the xylem appears to be practically identical with that of *M. Sutcliffei* and *M. Lomaxii*, in that scalariform tracheides are limited to the leaf-traces or their immediate neighbourhood, and differs from that of *M. poroxylodes* and *M. multirame*, in which there is everywhere a considerable thickness of spiral and scalariform elements before the typical pitted tracheides of the secondary wood begin.

Phloem. In *M. platypodium* the phloem is not well preserved. It is narrow, forming a zone only about 0.2–0.3 mm. in width, bounded externally by a discontinuous band of dark contents-filled cells, shown in transverse section in Pl. XXVII, Fig. 13, *s.s.*, and in radial section in Pl. XXV, Fig. 3, *s.s.*

In transverse section the phloem exhibits, in places, some indication of radial rows of elements continuous with those of the wood; otherwise nothing can be seen but rounded cells, many of which appear to have dark contents. In longitudinal sections faint indications of long thin-walled elements are visible, with larger short cells (? phloem parenchyma) in vertical rows, similar to the cells forming the greater part of the outer pith. The dark external zone of the phloem appears to consist of long cells with very

dark contents ('secretory sacs'), and an arc of these cells is carried out with the trace bundles, and appears outside the phloem of each bundle, as shown in Pl. XXV, Fig. 2, *s'.s'*, and Pl. XXVII, Fig. 15, *s'.s'*. This is quite similar to what was described in *M. Sutcliffii*.

Pericycle. Outside the phloem is a zone of tissue which was described in the other species as the pericycle. It consists, as seen in transverse sections, of rounded cells without radial arrangement, and many of which have abundant brown contents, thus differing from the more empty-looking cells of the cortex outside it, as shown in Pl. XXV, Fig. 2, *pe*. The thickness of the pericycle is generally about 0.3–0.4 mm., but this becomes much greater at the places where a leaf-trace and the axillary steles are embedded in it, as shown in Pl. XXV, Fig. 2, *pe*., where the breadth is about 0.8–0.9 mm.

In longitudinal sections the pericycle tissue is seen to consist mainly of rounded cells about 0.05 mm. in diameter, with dark contents mostly contracted towards the centre (Pl. XXV, Fig. 3, *pe*). The cells are similar to those of the pith in size and appearance, excepting that the contents are more dense, and they are not arranged in vertical rows, excepting, possibly, where they pass outwards into the cortex.

Besides these cells, the pericycle also contains scattered very long and narrow elements with very dark contents, which can sometimes be followed for a length of over 3 mm. in the vertical sections. These long sac-like elements are possibly of a secretory nature ('resiniferous tubes'), and are similar to those observed in the phloem and pericycle of *M. Lomaxii*, *M. Sutcliffii*, and other species.

Cortex. The cortex exhibits the structure common to the genus, with more or less radial bands of sclerotic tissue often extending inward in this form to the outer edge of the pericycle (Pl. XXVII, Fig. 15, *s.c.*). These bands sometimes anastomose, as seen in the transverse sections. No periderm formation is shown, probably because the stem is young. Longitudinal sections show that the sclerotic bands pursue a long nearly parallel course in the vertical direction, joining at intervals (Pl. XXV, Fig. 3, *s.c.*). Between these bands the cortex consists of empty thin-walled cells, which in longitudinal sections are seen to be short and broad and in definite vertical rows.

Outside the *Sparganium* zone there is a hypodermal layer of smaller somewhat thickened cells (Pl. XXVII, Fig. 15, *h.*) which in vertical sections appear to be prosenchymatous. The sclerotic bands do not extend into this zone, but it is continuous with, and graduates into, the thinner-walled tissue between them.

Outside the hypodermis is often seen a well-preserved epidermis (Pl. XXVII, Fig. 13, *e.*).

The following is a summary and diagnosis of this species, slightly

modified and expanded from that given in our Preliminary Note (6, p. 239) *Mesoxylon platypodium*, Scott and Maslen :

Leaf-bases very broad, scattered.

Pith large, with a persistent outer zone. Interior not preserved.

Twin-bundles of each leaf-trace at the pith margin very far apart (about 2 mm.), converging very slowly downward. Where they leave the pith margin to pass outward, each of the bundles is already divided, as regards its primary (centripetal) xylem. No bundle sheath developed.

Leaf-trace in pericycle and cortex consisting of two distinct rows of four bundles each.

Centripetal xylem persistent at the margin of the pith for some distance downward, but dying out while the bundles are still widely separated.

Two distinct axillary steles to the same leaf.

Tracheides of secondary xylem mainly pitted, passing inward through transitional tracheides into a narrow zone of scalariform ones. Secondary xylem sometimes pitted throughout. Scalariform tracheides almost confined to the leaf-traces or their immediate neighbourhood.

Medullary rays 1-14 cells in height.

Sparganum zone of cortex narrow.

Roof-nodule; Shore, Littleborough, Lancashire. Lower Coal-Measures.

MESOXYLOIDES PLATYPODIUM, gen. and sp. nov.

Many years ago, Dr. D. H. Scott obtained from Mr. Lomax a series of transverse and longitudinal sections of a stem which is obviously very similar to *Mesoxylon*, and was at first referred to *M. platypodium*, on account of the wide separation of the bundles of the leaf-traces on the margin of the pith.

More detailed study of the slides, which were probably all cut from the same specimen, shows, however, that it differs from *Mesoxylon platypodium*, and indeed from the original diagnosis of the genus as a whole, in that the leaf-traces, in the upper part of their perimedullary course, and during their exit through the wood, consist of *three* bundles instead of two, and also from all known species of *Mesoxylon* in the large number of axillary steles in the pericycle and cortex, as many as five of these being seen corresponding to a single leaf-base, and there were probably more than this number. Other minor differences from *Mesoxylon platypodium* are also observed and will be described later.

An important character of all species of *Mesoxylon* is that the 'leaf-

traces are double where they leave the pith' (6, p. 239), and without dropping this character it is impossible to include a form with three-bundled perimedullary traces in the same genus.

As the new form is anomalous in this and some other respects, as compared with *Mesoxylon*, while in most of its general characters it is very closely similar to this genus, it will be described under the generic name of *Mesoxylodes*. The species name *platypodium* has been adopted, the same as that of *Mesoxylon platypodium*, because, in some important respects, including the great width of the leaf-base, the new form is nearer to *Mesoxylon platypodium* than to any other known species of that genus.

We now know, from Shore material, vegetative stems of *Mesoxylon* type in which the leaf-trace, where it leaves the pith margin and passes outward through the wood, is:

- (1) *Single*, as in *Mesoxylopsis* (5, p. 17).
- (2) *Double*, in *Mesoxylon* itself.
- (3) *Triple*, in *Mesoxylodes*.

The original slides of *Mesoxylodes*, now contained in the Scott collection at the Natural History Museum, are seven transverse sections (Nos. 2968–2970 and 2973–2976, inclusive) and two longitudinal ones (Nos. 2971, 2972). The original transverse sections fall into two series, in each of which it has been possible to determine the order of succession as follows:

<i>First Series.</i>	<i>Second Series.</i>
2968 (highest).	2974 (highest).
2969.	2976.
2970 (lowest).	2975.
	2973 (lowest).

Unfortunately, it has not proved possible to 'fit on' the one series to the other, as there is probably a considerable gap between them. Fortunately, it has been possible to secure the loan of a number of other transverse sections of the same form, which were probably cut from the same specimen. These sections have kindly been lent by Professor F. W. Oliver, F.R.S., Professor A. C. Seward, F.R.S., Professor D. M. S. Watson, F.R.S., and Professor Boyd Thomson of Toronto. The latter sections were kindly searched out for us by Professor W. T. Gordon, D.Sc., during a visit to Canada in 1926. These additional slides have been of great value in the study of *Mesoxylodes*.

The additional slides are as follows:

- Oliver Slide, No. Q 0506.
- Cambridge Slide, No. 542.
- Watson Slides, Nos. A 116, A 117.
- Boyd Thomson Slides, Nos. 177, 178, 179.

It has been possible to 'fit together' the First Series of original slides with the Watson and Boyd Thomson ones as follows:

Highest, A 117 (Watson).

178 (Boyd Thomson).

179 (Boyd Thomson).

2968

2969

2970

177 (Boyd Thomson).

Lowest, A 116 (Watson).

Thus we now have a 'run' of eight transverse sections, through which the same trace can be followed from section to section, and in all of which (excepting No. 179) the steles belonging to the same branch are visible. In No. 179 the part which would contain the steles is broken away.

The slides we have of *Mesoxyloides* represent a stem of greater size than *Mesoxylon platypodium*. It is very incomplete and flattened, but parts of both sides are shown. A portion of one side is shown in Pl. XXV, Fig. 4. It evidently had a very large pith, of which, however, only the outer part is preserved, so that in this form, as in *Mesoxylon platypodium*, it is impossible to determine whether the inner pith was discoid or not.

As in the latter form, the leaves were evidently widely spaced on the stem.

The Pith.

The outer zone of the pith (Pl. XXV, Figs. 4 and 5, *o.p.*), the only part preserved, forms a thin layer, about 0.3–0.4 mm. wide, showing marked differentiation into two zones: the inner part composed of empty-looking cells, while in the outer part, next the xylem, the cells are filled with dense contents. In this respect the persistent pith is in marked contrast with that of *Mesoxylon platypodium*, which shows very little differentiation, and is more like the outer pith in *M. Sutcliffii* (1, Pl. XXV, Fig. 3), *M. poroxyloides*, and *M. multirame*. Many of the cells show evidence of tangential and other division. Actually abutting on the xylem, between it and the dark elements, clear cells are often seen.

In longitudinal section the pith cells are seen to be in vertical rows, as in *Mesoxylon*, with the innermost ones somewhat larger (about 0.06 mm. in diameter) and short in the vertical direction, while the elements next the xylem are longer in the vertical direction, as in *M. Sutcliffii*, *M. poroxyloides*, and *M. multirame*.

The Leaf-traces.

In the consecutive series of eight transverse sections, what appears to be the same trace can be followed all through the series. In the lowest one, slide No. A 116, a definitely double bundle (Pl. XXV, Fig. 5, *d.b.*) is seen at the pith margin, with a tangential breadth of about 2 mm. from the outer

edges of the bundles, or about 0.8–0.9 mm. from centre to centre. There is very little, if any, intermediate wood between the individual bundles.

The amount of centripetal xylem, *c.p.*, in the individual bundles of the pair is small, and it is not clearly divided into two. A promontory of the pith occurs opposite this bundle pair.

At a distance of about 4 mm. from the nearer bundle of the pair is another bundle, *o.b.*, in a similar position on the edge of the pith and apparently in a similar phase, and opposite another slight promontory of the pith. Following the pith edge no other bundle is seen for a long distance. This bundle, although a single one as regards its centrifugal xylem, has its small amount of centripetal wood distinctly divided into two parts. Moreover, the tangential width of this 'odd' bundle is considerably greater than that of one bundle of the pair, though less (in proportion 3–2) than the pair taken together.

Thus, in this form, the leaf-trace, while still on the pith margin, and, as will be seen, considerably below the level at which it passes out, consists of *three* distinct bundles, two of which are closer together forming a pair, while the third (odd) one is widely separated from the others, the distance from the centre of the odd bundle to the centre of the pair being nearly 5 mm.

As will be shown later, the odd bundle remains a single one, except for the division of its centripetal xylem, until after it has traversed the woody zone of the stem.

The trace described can be followed *upward* through the eight consecutive sections, and the changes it undergoes will be described later, but, unfortunately, it cannot be traced *downward*. Another trace is, however, seen in this series of sections, consisting of two bundles only, and evidently representing a trace cut at a lower level, and before the division of one bundle into two had taken place. We may now follow this trace *downward* from the highest of the series of sections:

In slide No. A 117 the trace, consisting of two bundles, is seen with the bundles about 4 mm. apart (centre to centre), i. e. nearly as far apart as the extreme bundles of the three-bundled trace seen in No. A 116 described above. One of the bundles is slightly larger (broader tangentially) than the other, and in this one the centripetal xylem is more definitely separated into two parts, probably preparatory to the division of the bundle into two at a higher level, i. e. a level corresponding to that seen in No. A 116 (Pl. XXV, Fig. 5).

In the next (lower) section, No. 178, this trace is not preserved, but in No. 179 it is seen again, still with the bundles about the same distance apart. The bundles are now somewhat smaller, and only little centripetal xylem is present.

The next lower section (No. 2968) is unsatisfactory, but in No. 2969 the trace is again visible with much the same characters as in No. 179, and

still with the bundles about the same distance apart. In No. 2970 they are again visible, with very little centripetal xylem. In No. 177 they are similar, and in No. A 116 (the lowest of the series) the part which would contain them is not preserved.

Thus, all through the length represented by this series of slides, the trace bundles remain about 4 mm. apart, i. e. there is little or no evidence of convergence downward. A similar absence of convergence, or at least very slow convergence, of the two bundles of the trace has been noted above (p. 506) in *Mesoxylon platypodium*.

Summing up the characters of the trace in *Mesoxyloides platypodium* in its lower course while still on the pith margin:

- (1) The leaf-trace is composed at a low level of two bundles only.
- (2) These bundles—4–5 mm. apart—follow a nearly parallel course up the stem, and gradually acquire their centripetal xylem.
- (3) One of the bundles next becomes somewhat larger than the other, and in this one the centripetal xylem splits into two, preparatory to the division of the bundle as a whole (centrifugal as well as centripetal xylem) which takes place at a higher level, but while still on the margin of the pith.
- (4) The other bundle of the original pair also shows the division of its centripetal xylem, but as will be shown later no splitting of this bundle as a whole takes place until after its exit from the wood.

A certain degree of asymmetry has been noted above in the leaf-trace of *Mesoxylon platypodium* (p. 507), but in *Mesoxyloides* it is obviously very much greater; indeed, if the specimen is a normal one, it is remarkably so. It also differs from *Mesoxylon platypodium* in the much wider separation of the original bundles of the trace, which, in the latter form, is only 2 mm., compared with 4–5 mm. in *Mesoxyloides*. Another difference is the division of one of the bundles before leaving the pith margin into two complete bundles with both centripetal and centrifugal xylem, a division which is only initiated in *Mesoxylon platypodium* by the division of the centripetal wood, both bundles passing through the xylem ring, in that form, as single bundles. In no other species of *Mesoxylon* is there any sign of division of either centripetal or centrifugal xylem until after the escape of the bundles from the secondary xylem zone.

Thus, in *Mesoxyloides platypodium* the division of one of the bundles takes place at a much lower level than in any known form of *Mesoxylon*.

The *upward* course of the trace may now be followed from the level seen in slide No. A 116 (Pl. XXV, Fig. 5), through the series of eight transverse sections.

In the next (higher) slide, No. 177, the trace has undergone but little change, excepting that a small amount of intermediate xylem has appeared

between the bundles of the pair, indicating a slight divergence of the bundles from one another. The odd bundle is still a single one, although its centripetal xylem is clearly divided (as in the lower section).

The next section, No. 2970, also shows very little change, but the distance between the bundles of the pair is now slightly greater (1 mm.).

On Pl. XXVIII, Fig. 19, is shown the two bundles of the pair drawn from another section (Oliver slide, No. Q 0506), and representing part of a trace at about the level of the last two slides. Here, however, the primary xylem, x ., of one of the bundles (the right-hand one in the figure) is definitely divided into two.

In the next slide of the series, No. 2969 (Pl. XXV, Fig. 6), the trace bundles are definitely preparing to leave the pith margin. The centripetal xylems of the pair are now about 1.1 mm. apart (slightly greater than before), and the distance from the centre of the odd bundle to the middle of the pair is now over 5 mm. Moreover, one of the bundles has developed a bundle sheath, separating the xylem from the pith, and similar to that which forms a characteristic feature in *M. Lomaxii* (3, p. 1014) and *M. multirame* (4, p. 447), but which has not been observed in the other species of *Mesoxylon*, including *M. platypodium*. The sheath is formed by tangential divisions in the thin-walled, small-celled tissue, between the centripetal xylem of the bundle and the ordinary pith cells, as the bundle prepares to leave the pith margin, resulting in rows of cells radiating from the small group of centripetal xylem elements. But in this section only one of the three bundles shows a sheath (Pl. XXV, Fig. 6, *b.s.*), viz., the bundle of the pair distant from the odd bundle. This one is also, as we shall see, the first bundle to pass out.

Thus the want of symmetry, which is such a marked feature of this form, is seen in the development of the bundle sheath also, which is itself related to the fact that the bundles of a single trace pass out at somewhat different levels.

The following slide, No. 2968, shows a further stage of the outgoing trace. The bundles of the pair are now about 1.4 mm. apart, and one, the outer one, is considerably farther out than its fellow, and its bundle sheath is much better developed than in the section below. The inner bundle of the pair has not yet developed its sheath, although a few tangential divisions in the cells next the pith doubtless indicates the commencement of its formation. The odd bundle is again larger than either of the others, and has its centripetal xylem more definitely divided; it has now developed a sheath, correlated with the fact that it is the second bundle to pass out.

No definite reticulate markings can be seen in the sheath cells, such as were observed in *M. Lomaxii* (3, p. 1015).

The traces evidently follow an almost vertical course through the wood, as the bundles are cut almost transversely in transverse sections of the stem.

The next section, No. 179, shows the bundles still farther out (Pl. XXVI, Fig. 7); the outer one of the pair, with its well-developed sheath, *b.s.*, farther out than the other. Next comes the odd bundle, which also has a well-marked sheath; and lastly the inner one of the pair, which is still quite near the pith margin, and has only a weakly developed sheath. The distance between the bundles of the pair has now increased to nearly 2 mm., while that between the centre of the pair and the centre of the odd bundle remains about 5 mm.

In the next section, No. 178, shown in Pl. XXVI, Fig. 8, the outer bundle of the pair has reached the external edge of the wood, which has now closed in behind it; its sheath has lost its distinctiveness, and is apparently merged in the secondary xylem. At this level the inner bundle of the pair, although farther out than in the section below, is still near the pith margin; its bundle sheath has become somewhat better developed, but is still quite small compared with those of the other bundles. The odd bundle, *o.b.*, is now nearly half-way through the wood, and its sheath, also, is becoming merged in the secondary wood, which is closing in behind it. Elements similar to those of the outer pith, including some of the cells with dense contents, accompany the bundles outward.

The next section, No. A 117, the highest of the series of eight slides, shows that two of the three bundles have now escaped from the wood (Pl. XXVI, Fig. 9). One, the outer one of the pair, *b.p.o.*, is now well beyond the outer edge of the wood; the odd bundle, *o.b.*, is just escaping from it; while the inner one of the pair, *b.p.i.*, is about midway through, following its fellow almost in a straight line, probably owing to slight lateral displacement of the tissues along the outer edge of the xylem.

The odd bundle is somewhat wider than the other escaped bundle, and its centripetal xylem is in widely separated masses, probably indicating that it will be the first bundle to divide in the pericycle. The outer one of the pair also has its centripetal xylem divided, but the distance between the two groups is much less. There is no evidence that the primary xylem of the inner bundle of the pair has yet divided.

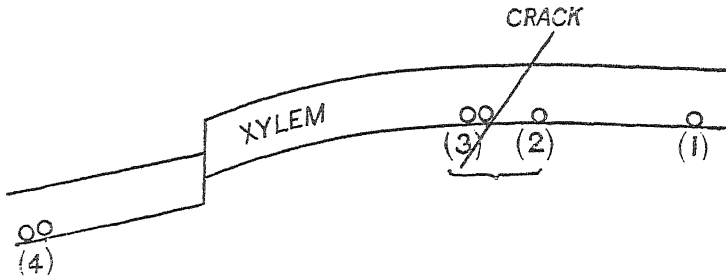
Unfortunately this particular trace cannot be followed to a higher level, so that the further division of the three bundles which thus escape from the wood at different levels cannot be seen. One of these sections, the lowest, No. A 116, does, however, show a trace in the cortex, which will be described later.

The only two longitudinal sections we have of this form do not pass through any leaf-trace bundles passing through the wood, or in the tissues outside it.

We may now consider the evidence afforded by the other series (Second Series) of the original sections of *Mesoxyloides platypodium* (Nos. 2973-2976), as to the behaviour of the traces on the margin of the pith and in

their exit through the wood. The order of succession has been determined and is given on p. 516.

Slide No. 2973, the lowest of the series, shows a long, almost straight transverse section of the stem about 5 cm. in length, which evidently represents only a portion of the whole stem.



TEXT-FIG. 1. Rough sketch of xylem showing relative position of leaf-trace bundles on margin of pith. Slide No. 2973.

The rough sketch of the xylem, Text-fig. 1, shows the relative position of the well-defined bundles in this section, all of which are at or near the pith margin.

Starting from one end of the section we see a single bundle (1) about 1.3 mm. in greatest tangential width. It is opposite a slight inward bulge of the pith, and its centripetal xylem is clearly divided, the separation being about 0.4 mm. It shows no indication of going out and no sheath; and as the distance between it and the next bundle (2) is 7–8 mm., it is probably the odd bundle of a trace of which the pair is lost.

Bundle (2) is another single one of about the same size as (1), but with rather less centripetal xylem, the main part of which is divided into two portions, which are, however, connected by a line of small tracheides. It probably represents part of a trace which would go out at a higher level than (1). The distance between it and the centre of the next pair (3) is about 5 mm., corresponding to the distance between the extreme bundles of the trace described in the other series of slides. All three bundles (2 and 3) are in much the same phase, lie on the edge of the pith, and show no indication of going out or of the development of a sheath. We probably see here another example of the three-bundled trace in the upper part of its perimedullary course, consisting of a bundle-pair separated widely from the third (odd) bundle. Moreover, as described in the other trace, while the odd bundle has its centripetal xylem clearly divided, no clear division can be seen in the individual bundles of the pair.

The distance, centre to centre, between the bundles of the pair is about 1.3 mm., and about 4–5 radial rows of intermediate tracheides occur between them. This trace appears to correspond to a level between that seen in slides Nos. 2970 and 2969 of the other series, previously described.

Beyond the bundle pair (3) for a long distance—2.5 cm., to and beyond the 'fault' shown in the diagram—no clearly defined bundles with centripetal xylem are seen, although faint indications of bundles cut far down, after the disappearance of the primary xylem, are visible.

At the end of the section appears the double bundle (pair) marked (4) in the Text-fig., which probably represents part of another trace, the odd bundle of which is lost. Both bundles are definitely going out, one slightly farther out than the other. Both possess the sheath, and neither has its centripetal xylem completely divided, although there is a suggestion that separation is beginning. The separation of the two bundles is about 2 mm.

We now proceed to follow these traces upward, as far as possible, through the three succeeding slides of this series :

Bundle (1) is not preserved in any of the other sections. The trace consisting of the single bundle (2) and the double one (3) can be followed through all of them.

In No. 2975 it shows but little change as compared with the section below. The odd bundle (2) is somewhat wider, and the connecting line of small tracheides between the two centripetal xylem groups has disappeared. The pair of bundles (3) is also much the same, excepting that the intermediate wood between the bundles is now much broader, and the distance between the bundles consequently greater (about 1.7 mm.). No sheath is yet developed, and no separation of the centripetal xylem is visible. The distance between the pair and the odd bundle is now just over 5 mm.

In the next slide, No. 2976, there is again but little change, but the distance between the bundles of the pair is still a little greater.

The next and highest section, No. 2974, shows the trace again, still not definitely going out, but preparing to do so, as indicated by the beginning of sheath formation inside the odd bundle, especially on one side of it. This bundle now has a width of nearly 1.8 mm. (wider than at a lower level), while the distance between its well-developed centripetal xylem groups is 0.5 mm. In the pair the distance between the bundles has now increased to about 2 mm. No sheath has formed to either of the bundles, and there is no absolute separation of the centripetal xylem.

Thus, as far as it goes, the study of this trace confirms the observations made on the corresponding portion of the trace in the other series of slides.

Passing now to the bundle pair (4), probably representing part of another trace, and which in No. 2973 is preparing to go out, as described above. In the next section, No. 2975, what appears to be the same trace is again seen in a damaged condition opposite a broad bay in the pith. One of the bundles is now on the outer margin of the wood ; the other one is not clearly seen, but it is evidently not nearly as far out.

In slide No. 2976 both bundles have apparently passed out, and one of them is visible just outside the wood.

In the next and highest section, No. 2974, nothing can be seen of this trace.

If the interpretation of the traces seen in these slides, as given above, is correct, it would appear that there is irregularity in the order in which the respective bundles pass out—another indication of the remarkable asymmetry of this form. In the first trace described the order was: first, the outer one of the pair; then the odd bundle; and, lastly, the other (inner) one of the pair. In the trace partly represented by (4) in Nos. 2975–6 the bundle which appears to be the inner one of the pair passes out before the other, while in the complete trace (2 and 3) shown in No. 2974, judging from the development of the sheath on the odd bundle before it appears on the pair, the odd bundle would be the first to leave the pith margin. Also, if the interpretation is correct, the odd bundle is on opposite sides of the pair in successive traces.

There remains for description the one section in which a leaf-trace is preserved in the cortex. This is No. A 116, the lowest of the series of eight sections. Unfortunately, this trace is not preserved in any of the other slides.

In this section the trace is seen, together with two small steles. It shows (Pl. XXVI, Fig. 10) three smaller bundles, and also three broader ones; in each of the latter the centripetal xylem is more or less divided into two, probably indicating that each of these would divide into complete bundles at a slightly higher level, giving nine bundles. It is not improbable that one or more bundles are lost. The tangential width of the smaller bundles is 0.4–0.5 mm., and that of the wider ones 0.8–1.0 mm., so that these bundles are considerably smaller than their parent ones, where just free from the xylem.

It seems that the leaf-trace in *Mesoxylodes platypodium* may have consisted of more than eight bundles. In most species of *Mesoxylon* the number of bundles of a trace in the pericycle and cortex appears to be eight, but further division must take place in some species, as in *M. Sutcliffii*, in which the flattened petiole, near its insertion on the stem, has been seen to possess about sixteen bundles (l, p. 405, and Pl. XXXV, Fig. 18).

Returning to the trace seen in No. A 116, the trace as preserved (and some bundles may be lost) stretches over a great tangential width, over 1 cm., which is not much greater than in *Mesoxylon platypodium*, in which the entire trace of eight bundles measures 0.8 mm. from end to end.

The Axillary Steles.

As already mentioned, a remarkable feature of *Mesoxylodes platypodium* is the large number of axillary steles in the pericycle and cortex, as many

as five having been observed (and there were probably more) corresponding to a single leaf-trace.

A review of our knowledge of the axillary steles in species of *Mesoxylon* is given in an earlier part of this paper (p. 509). As there shown, a special character of *M. platypodium* is the presence of two widely separated steles in the pericycle and cortex corresponding to a single leaf. Some evidence has also been given that, in spite of the wide separation, the two steles may fuse into one before actual entry into the bud or branch—a fusion which was demonstrated in *M. Sutcliffii*, in which there are occasionally twin bud steles in the pericycle and cortex.

In the sections of *Mesoxyloides* the steles are shown, more or less completely (the preservation is as usual patchy in these roof-nodule plants), through the long series of eight transverse sections. Nothing is known of the origin of the steles, or of their passage through the wood, as, in the lowest of the sections, the steles are well outside the xylem in the pericycle.

In this lowest section, No. A 116, two small steles are seen inside the subtending leaf-trace (Pl. XXVI, Fig. 10, s.s.), about 6–7 mm. apart. As there is some disturbance in the tissues between, this may not be the original distance. The corresponding distance in *Mesoxylon platypodium* (a smaller stem) is only 3 mm. As in the species of *Mesoxylon*, in which axillary steles have been observed, they are elongated tangentially, the best preserved one measuring about 1.7 mm. by about 0.8 mm. (or across xylem only, about 1 mm. by 0.5 mm.). This is considerably larger—more than double the size—of the steles in the corresponding position in *Mesoxylon platypodium*. The steles are cut nearly transversely, indicating an almost vertical course. The structure, as far as can be determined, is quite typical of that of the steles of *Mesoxylon*, as illustrated by *M. Sutcliffii* (1, Pl. XXXIV, Fig. 11). Outside the xylem is a broad space representing the position of the phloem and pericycle. Each of these steles is subtended by a group of trace bundles, as shown in Pl. XXVI, Fig. 10.

But closer examination of this section reveals evidence of two other steles, both on the tangentially outer side of one of the main ones; these steles are almost destroyed, the xylem wholly so, but traces of the outer tissues are preserved. These steles (s'.s') are small, about 0.5 mm. across. One is quite close to the main stele, but the other is widely separated from it by a distance of 3–4 mm. There may have been yet other steles. Both these steles are on the outer side of the same main stele; the other main stele shows no sign of having divided. In the stretch of 3 mm. of well-preserved cortical tissue between it and the end of the section there are no steles, although it is not impossible, judging from the distance (3–4 mm.) of the more distant of the two subsidiary steles from the other main one, that a stele may have been present off the end of the section. Even as

preserved, the steles are seen stretching over an arc of the stem, which measures nearly 1.5 cm. Thus at this level there is evidence of at least four steles.

In the next (higher) section, No. A 177, the same two main steles are seen, slightly larger and somewhat farther out. The subtending trace is not preserved in this or in any of the higher sections. This section is much broken, and no other steles can be seen. In one of the steles the phloem is partly preserved, and shows radial rows of thin-walled elements separated by rows of larger ray cells, which, as described in *M. multirame* (4, p. 443), are often dilated tangentially. Beyond the phloem is a band of thin-walled tissue in which the cells are not radially disposed, and which is presumably the pericycle, and this is bounded by the still larger-celled tissue (many of the cells with dark contents) of the cortex of the main axis.

The next section, No. 2970, again shows the two main steles, but still farther out. They are longer tangentially (over 2 mm.), and thus appear more flattened radially. One subsidiary stele only is visible, on the outer side of the same main stele as that which has two in No. A 116, and this is separated about 2 mm. from its main stele. It may represent the closer of the two small steles in the lower section, but no trace of the more distant one is visible, possibly due to non-preservation, as an empty space is seen in the tissues where it might be expected to occur.

The next section is No. 2969, and here considerable changes are apparent (Pl. XXVI, Fig. 11), although the actual number of steles visible is the same as in slide No. A 116. Two main steles, *m.s.*, still occupy much the same positions as in the lower sections, but are somewhat farther out. Each is accompanied by a small subsidiary stele, *s.s.*, one on the outer side of one main stele, and the other on the opposite side of the other. These small steles do not appear to correspond to any of the subsidiary steles shown in the lower sections. The main steles have become very long and narrow, elongated to 3.5 mm., in the tangential direction, while remaining much the same as before (nearly 1 mm.) radially. The small steles are nearly round in section and measure 0.7–0.8 mm. across.

It seems impossible to correlate the steles in these two sections (Nos. 2969, 2970): the appearance is as though the steles divide and join up again in an irregular manner. The total spread of the steles remains much the same as before.

The next section, No. 2968, is peculiar again. Corresponding in position to one of the long steles and its accompanying small one in the slide below there are now three steles quite close together—one large one and two small ones, the middle one being exceedingly small. The small steles are on the inner side of the largest one, i.e., on the opposite side to the smaller stele in the section below. Correlation of the steles in these two sections is also impossible, and we seem to have in this slide further evidence

of the erratic way in which the steles in this form divide and join up again. On the other side two moderate-sized steles are visible in place of the one in the section below; as one of the two steles is at the end of the section there may have been more. Thus, in this section there are *five* visible steles, and there may have been more.

In the next slide, No. 179, the part which should show the steles is lost, and in the succeeding one, No. 178, all that is visible is a portion of one large stele at one end of the section. This stele has the usual structure, excepting that the ring of xylem appears to be open on its inner side. On the outer side there are the usual loose radial rows of xylem elements (about six or more in each row), the tracheides becoming gradually smaller inwards. Traced round the end of the flattened stele the xylem maintains its thickness, but on the inner (axial) side the number of elements in a row diminishes to five, four, two to none, so that the pith of the stele becomes continuous with the tissue outside the xylem.

It will be remembered that a quite similar 'open' stele was observed in *Mesoxylon platypodium* (p. 512, and Pl. XXVII, Fig. 18), in which form there are two steles only, and it was suggested that it represents a stage in the opening out and fusion of the two steles coming up from below before entry into the bud or branch. It seems probable that in this case it represents much the same thing. In *M. Sutcliffei* also (in which two steles are sometimes present) there is evidence of fusion as they pass upwards and outwards. It is impossible to determine whether the stele observed in slide No. 178 is a stage in the fusion of two of the several steles coming up from below, or whether it represents the formation of the final single stele.

The next and highest slide of the series, No. A 117, shows part of a stele in much the same position as that seen in No. 178, but still farther out in the cortex; in fact, approaching its outer edge. This stele (Pl. XXVI, Fig. 12) is large, and very much elongated in the tangential direction, the part preserved, to the end of the section, measuring 3 mm. It evidently represents parts of a stele considerably larger than any other observed. This stele is interesting on account of the marked difference between its inner and outer sides. On the *outer side*, *o.*, the tissues are well developed and well preserved; the structure resembles that of the main stem without the secondary xylem between and outside the bundles, and is quite different from that of the axillary steles farther in. There are indications of the development of a differentiated pith, *p.*, like that of the main axis, a narrow zone of cells with dark contents occurring near the xylem, passing inward into other pith elements which are either empty or have much paler contents. On this outer side also, the xylem, *x.*, consists of long radial rows of about ten elements, only the inner three or four of which have thickened walls; moreover, the xylem has the appearance of

being definitely aggregated into bundles, similar to the centrifugal portions of the trace bundles on the margin of the pith of the main stem.

On the *inner side*, *i.*, there is no indication of pith differentiation, the xylem is much less developed, much more loose and scattered, with no indication of arrangement in bundles, and in one place thinning away to nothing, thus leaving a gap in the xylem ring, *g.*, 0.3–0.4 mm. in length. Thus this stele presents the open character of that described in the section below (No. 178).

The probability is that this stele does represent a stage in the final fusion of the steles into one. From its markedly elongated form when quite near the outside of the stem, it seems possible that it may have passed into a flattened shoot, something like that which forms such a characteristic feature of *Mesoxylon multirame* (4, p. 442).

The following is a summary of our conclusions on the axillary steles of *Mesoxylodes platypodium* from the study of this series of slides:

1. Probably two axillary steles passed out from the wood, as in *Mesoxylon platypodium*, but at a much greater distance apart, corresponding to the much greater separation of the bundles of the trace in this form. The bundles of the subtending leaf-trace are in groups, and a group sometimes subtends an individual large stele.
2. These primary steles divide and reunite in an irregular manner as they pass upward and outward, so that in some transverse sections of the stem as many as five steles of varying sizes are seen, and there may have been more.
3. Finally, before leaving the cortex of the main axis, the numerous steles join up into one larger one ready for its entry into the bud or branch.
4. The steles, like the leaf-traces, must ascend very steeply.
5. In spite of the fact that the steles stretch over an arc of the stem of at least 1.5 cm., they must correspond to one leaf-base, as they are closely related to the leaf-trace seen in slide No. A 116. Moreover, it is out of the question for two leaf-bases to be close together when there is evidence that the leaves were not crowded but widely spaced.

The difference from *Mesoxylon platypodium*, in addition to the greater distance between the primary steles, is the division of the original steles in the pericycle and cortex.

The Xylem, Phloem, Pericycle, and Cortex of the Main Axis.

Only brief notes on these tissues will be given, as they are generally similar to those of the species of *Mesoxylon*.

Xylem. The ring of centrifugal xylem is of the type characteristic of

Mesoxylon. The thickness of the woody zone is about 3-4 mm., more than double the thickness in *Mesoxylon platypodium*.

The only two longitudinal sections we have of *Mesoxylodes platypodium* are much alike, both more or less radial, showing wood on both sides of the pith, and cortex on one side detached from the wood. The pits on the radial walls are very commonly in three rows, sometimes two; they are usually crowded and irregularly alternating. The medullary ray cells have conspicuous, large pits.

The pitted tracheides pass inward, as in *Mesoxylon*, into scalariform elements abutting on the pith. Commonly about 4-5 scalariform tracheides occur between the pith and the pitted elements, and good examples of transitional tracheides are seen (slide No. 2971), in which the inner part of the radial wall is 'barred' while the outer part is definitely pitted. Occasionally an element with pits abuts directly on the pith.

In general the zone of reticulated tracheides is broader than in *Mesoxylon platypodium*, in which the pitted elements approach much nearer to the pith edge, although occasionally the thickness is greater, probably owing to the section passing across the centrifugal wood of the lower part of a trace. In this respect *Mesoxylodes platypodium* more resembles *Mesoxylon multirame* and *M. poroxylodes*, in which forms there is everywhere a considerable thickness of scalariform elements within the typical pitted tracheides, although the actual number of these elements in *Mesoxylodes* appears to be less.

Phloem. The phloem is very poorly preserved in all our sections of this form, and its structure cannot be described.

Pericycle. This tissue is better preserved and is of the usual type (Pl. XXV, Fig. 4, *pe.*). It is much thicker than in *Mesoxylon platypodium*, having a breadth up to about 1 mm., even when far from a leaf-trace or the steles. Long elements ('resiniferous tubes') occur scattered through this tissue, similar to those described in *Mesoxylon platypodium*; they are, perhaps, rather more abundant in the inner part of the pericycle than in its outer part, but they do not form the definite band just outside the phloem seen in *Mesoxylon platypodium*, nor do the trace bundles after their escape from the wood show the distinct arcs of cells with black contents which form a striking feature in those of the latter form. The pericycle is, as usual, much broader where the steles or a trace are embedded in it (Pl. XXVI, Fig. 10, *pe.*).

Cortex. The cortex (Pl. XXV, Fig. 4, *s.c.*) does not appear to show any special characters, excepting that there is no clear evidence of the hypodermal layer mentioned above in *Mesoxylon platypodium*. There is a well-marked epidermal layer.

The following is a diagnosis of the new genus:

Mesoxylodes, gen. nov.

Pith relatively large. Probably discoid, but not proved.

Wood dense of Cordaitean type, with narrow uniseriate medullary rays.

Leaf-traces *triple* where they leave the pith, dividing into eight or more bundles in the pericycle and cortex.

Centripetal xylem present in the leaf-traces at the margin of the pith and throughout their outward course into the leaves.

Cortex relatively narrow, with *Sparganium* zone.

Greater part of the secondary (centrifugal) xylem composed of pitted tracheides. Scalariform or spiral tracheides form the leaf-traces.

All the above characters, with the exception of the triple leaf-trace, are those of *Mesoxylon* (6, 1910, p. 237).

The following may be regarded as specific characters, in addition to those given for the genus:

Mesoxylodes platypodium, sp. nov.

Leaf-bases very broad; scattered.

Outer pith differentiated; outer cells (next xylem) filled with dense contents. Interior pith not preserved.

Each leaf-trace, when it leaves the pith, consisting of a pair of bundles separated about 2 mm., and an odd bundle widely separated from the others. The whole trace extends over about 5 mm. (from centre of pair to centre of odd bundle).

Primary (centripetal) xylem of each bundle divided into two before leaving the pith margin.

Trace bundles at the margin of the pith following a nearly parallel course for a considerable distance down the stem.

Each bundle develops a bundle sheath where it leaves (or approaches) the pith margin.

The three bundles of a trace pass out through the wood at somewhat different levels.

Axillary steles probably two in number when they leave the xylem ring and widely separated. These steles divide and reunite in an irregular manner in the pericycle, finally probably joining up to form one large stele in the cortex before entering the bud or branch.

Roof-nodule: Shore, Littleborough, Lancashire. Lower Coal Measures.

SUMMARY.

The structure of two stems, *Mesoxylon platypodium* (Scott and Maslen) and *Mesoxylodes platypodium* (gen. et sp. nov.), from the Lower Coal Measures of Lancashire, is described in detail and is summarized in the diagnoses given on p. 515 and on pp. 529, 530. The genus *Mesoxylon* was founded in 1910 for a number of forms referred to the Cordaiteae and

considered to be the last link in a chain of fossils connecting the Pteridosperms with the typical *Cordaites*. *M. platypodium* is the only form not hitherto described in detail. Outstanding characters of the species are the wide separation (about 2 mm.) of the paired leaf-trace bundles on the margin of the pith, the division of the centripetal xylem of each bundle before leaving the pith margin, and the presence of two axillary steles (corresponding to a single leaf) in the pericycle and cortex. A new genus, *Mesoxylodes*, is made for the reception of a stem evidently closely related to *Mesoxylon*, but differing from it by the possession of three-bundled asymmetrical perimedullary leaf-traces. The specific name, *platypodium*, has been given because in some of its general characters, including the great width of the leaf-bases, it is nearer to *Mesoxylon platypodium* than to any other species of that genus. Detailed comparison, however, shows that it differs from *Mesoxylon platypodium* not only in the number of bundles in the leaf-traces and in the large number of axillary steles, but also in the much wider separation (4 mm.) of the trace bundles even when far down the axis, the early division of both centripetal and centrifugal xylem of one of the original bundles, which takes place at a level considerably below that at which it leaves the pith margin, and in a number of minor characters. These differences make it improbable that it is an abnormal specimen of *Mesoxylon platypodium*, as it was at first thought it might be.

In conclusion, I wish to give my grateful thanks to Dr. D. H. Scott, F.R.S. It was originally intended to publish the results of this work under our joint-names, and a considerable amount of the preliminary work was done together. Dr. Scott then, very generously, passed over the whole of the material to me for completion. Thanks are also due to Prof. Boyd Thomson, Prof. D. M. S. Watson, F.R.S., Prof. F. W. Oliver, F.R.S., and Prof. A. C. Seward, F.R.S., for the loan of sections (to Dr. Scott) of *Mesoxylodes platypodium*, which have proved of great value in elucidating the structure of that form.

The photographic illustrations are the work of Mr. W. Tams, of Cambridge, and Mr. J. Rhodes, of the Geological Survey. The drawings have been made by Miss G. C. Harrison, Mr. G. T. Gwilliam, F.R.A.S., and the author.

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EXPLANATION OF PLATES XXV-XXVIII.

Illustrating Mr. Maslen's paper on *Mesoxylon* and *Mesoxyloides*.

Some of the photographs should be examined with a lens.

PLATE XXV.

Photographs by Mr. W. Tams and Mr. J. Rhodes.

Mesoxylon platypodium.

Fig. 1. General transverse section of stem. *o.p.*, outer pith; *x.*, xylem; *c.*, cortex; *pe.*, pericycle; *lb.*, protuberance of leaf-base. × 4. Slide No. 2385 (all slides without special designation are in the Scott Collection at the Natural History Museum, London).

Fig. 2. Part of transverse section showing outer portion of secondary xylem ring, phloem, pericycle, and part of inner cortex. In the locally thickened pericycle is seen one of the pair of axillary steles, and three of the eight bundles of the subtending leaf-trace. *s.x.*, secondary xylem; *pe.*, pericycle; *c.*, cortex; *lt.*, *lt.*, *lt.*, leaf-trace bundles; *a.s.*, axillary stele; *s.s.*, cells with dark contents ('secretory sacs') between phloem and pericycle; *s'.s'*, arc of dark cells carried out by leaf-trace bundles. × 45. Slide No. 2385.

Fig. 3. Longitudinal radial section of stem. *o.p.*, outer pith; *x.*, xylem; *p.t.*, pitted tracheides; *m.r.*, medullary rays; *p.*, phloem; *s.s.*, dark elements between phloem and pericycle; *pe.*, pericycle; *s.c.*, *Sparganium* zone of cortex. × 33. Slide No. 2388.

Mesoxyloides platypodium.

Fig. 4. Part of transverse section of stem. *o.p.*, outer pith; *x.*, xylem; *pe.*, pericycle; *s.c.*, *Sparganium* cortex; *lt.*, *lt.*, *lt.*, leaf-trace bundles. × 5. Slide No. A 117 (Watson).

Fig. 5. Showing the three bundles of a leaf-trace on the pith margin. *d.b.*, double bundle; *o.b.*, odd bundle; *c.p.*, centripetal xylem; *o.p.*, outer pith. × 18. Slide No. A 116 (Watson).

Fig. 6. The same trace beginning to pass out. *b.p.*, *b.p.*, the two bundles of the pair; *o.b.*, odd bundle; *b.s.*, bundle sheath. × 12. Slide No. 2969.

PLATE XXVI.

Photographs by Mr. J. Rhodes.

Mesoxyloides platypodium.

Fig. 7. The same trace as that shown on Pl. XXV, Fig. 6, but at a somewhat higher level. *b.p.*, *b.p.*, bundles of pair; *o.b.*, odd bundle; *b.s.*, *b.s.*, bundle sheaths. × 12. Slide No. 179 (Boyd Thomson.)

Fig. 8. The same trace at a still higher level. *b.p.o.*, *b.p.i.*, outer and inner bundles of pair; *o.b.*, odd bundle; *b.s.*, bundle sheath. $\times 12$ Slide No. 178 (Boyd Thomson).

Fig. 9. The same trace at a level when two of the three bundles have passed through the zone of secondary xylem. *b.p.o.*, *b.p.i.*, bundles of pair; *o.b.*, odd bundle; *c.p.*, *c.p.*, centripetal xylem of odd bundle. $\times 12$. Slide No. A 117 (Watson).

Fig. 10. Transverse section showing leaf-trace and axillary steles in pericycle. *l.t.*, leaf-trace bundles; *s.s.*, main steles; *s'.s'*, position of other steles; *pe.*, pericycle. $\times 8$. Slide No. A 116 (Watson).

Fig. 11. Transverse section showing four axillary steles. *m.s.*, main steles; *s.s.*, subsidiary steles. $\times 8$. Slide No. 2969.

Fig. 12. Transverse section of part of much elongated axillary stele in cortex. *o.*, outer side; *i.*, inner side; *p.*, pith of stele; *x.*, xylem; *g.*, gap in xylem ring. $\times 40$. Slide No. A 117 (Watson).

PLATE XXVII.

Drawings by Miss G. C. Harrison, Mr. G. T. Gwilliam, F.R.A.S., and the author.

Mesoxylon platypodium.

Fig. 13. Transverse section of part of stem. *o.p.*, outer pith; *x.*, xylem; *l.t.*, *l.t.*, the two bundles of a trace on the margin of the pith; *s.s.*, cells with dark contents between phloem and pericycle; *pe.*, pericycle; *c.*, cortex with bands of sclerenchyma (*Sparganum* zone); *e.*, epidermis. $\times 20$. Slide No. 2384.

Fig. 14. Transverse section of one of a pair of leaf-trace bundles at the margin of the pith. *x.*, primary (centripetal) xylem of bundle; *x.*, secondary (centrifugal) xylem of bundle; *p.x.*, protoxylem; *m.r.*, medullary ray. $\times 100$. Slide No. 2385.

Fig. 15. Transverse section of part of stem showing eight bundles of a leaf-trace in the pericycle. *s.g.*, stelar gaps in xylem ring; *s.c.*, *Sparganum* zone of cortex; *h.*, hypodermal layer; *s'.s'*, arc of 'secretory sacs' carried out by bundle; *i.x.*, intermediate xylem between the stelar gaps; *l.t.*, one of the bundles of the leaf-trace. $\times 8$. Slide No. 2384.

Fig. 16. Longitudinal tangential section passing through xylem and outer pith, and intersecting an axillary stele on the inner edge of the xylem ring. *o.p.*, outer pith with cells in vertical rows; *t.*, tortuous interlacing tracheides of axillary stele; *i.x.*, intermediate xylem between the steles (only one of which is shown in the drawing); *x.*, centrifugal xylem and medullary rays. $\times 40$. Slide No. 2391.

Fig. 17. Some of the interlacing tracheides of the axillary stele on a larger scale. *m.r.*, medullary ray cells. $\times 90$. Slide No. 2391.

PLATE XXVIII.

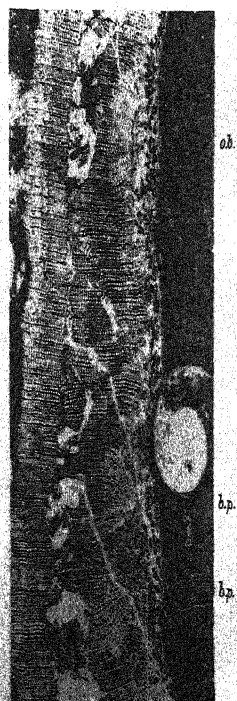
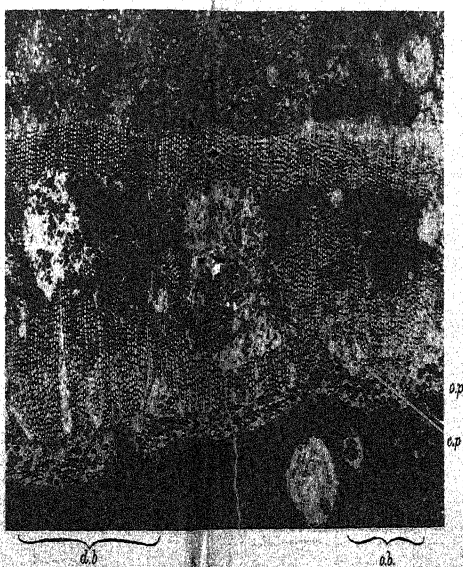
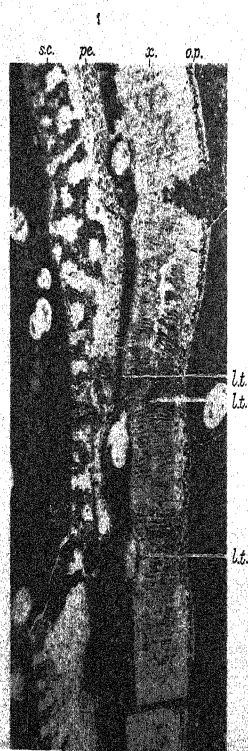
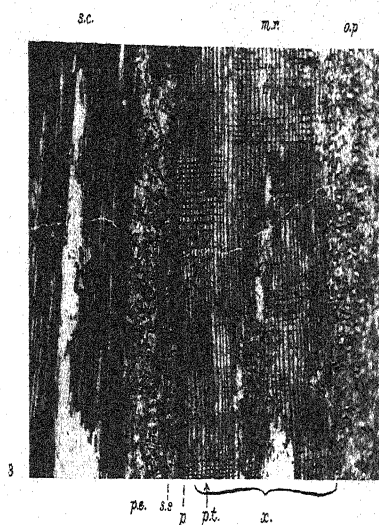
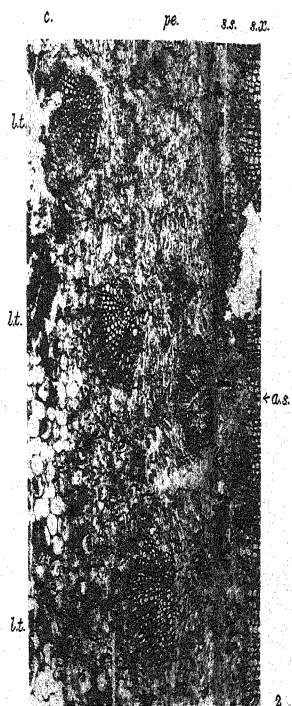
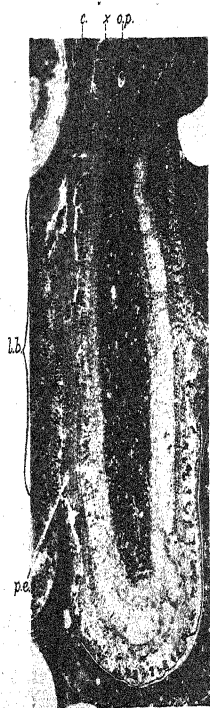
Drawings by Mr. G. T. Gwilliam, F.R.A.S., and the author.

Mesoxylon platypodium.

Fig. 18. Slightly oblique transverse section of axillary stele showing its 'open' character on the inner (axial) side. *t.*, tracheides; *p.*, pith cells; *pe.*, pericycle cells; *a.*, axial side of stele. \times about 120. Slide No. 2384.

Mesoxyloides platypodium.

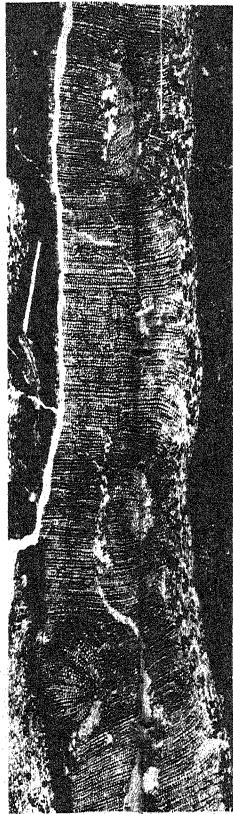
Fig. 19. Transverse section of part of a leaf-trace on the margin of the pith, showing the two bundles of the pair. *x.*, *x.*, primary (centripetal) xylem; *x'*, *x'*, secondary (centrifugal) xylem of bundles; *p.x.*, *p.x.*, protoxylem; *m.r.*, medullary ray. \times about 50. Slide No. Q 0506 (Oliver).



b.s.



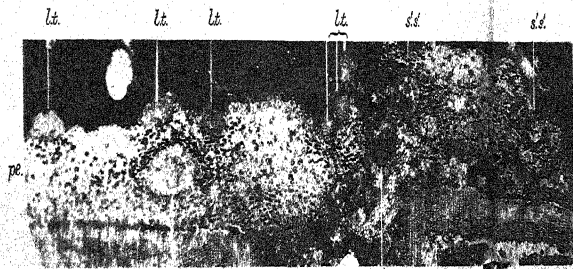
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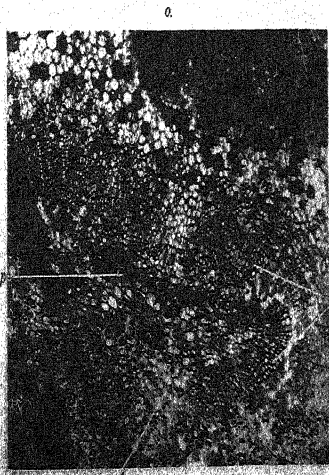
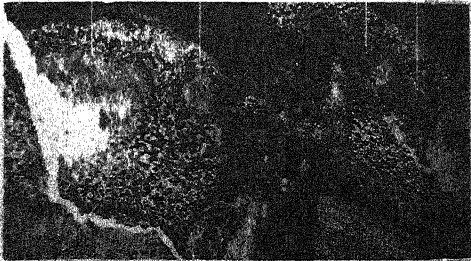
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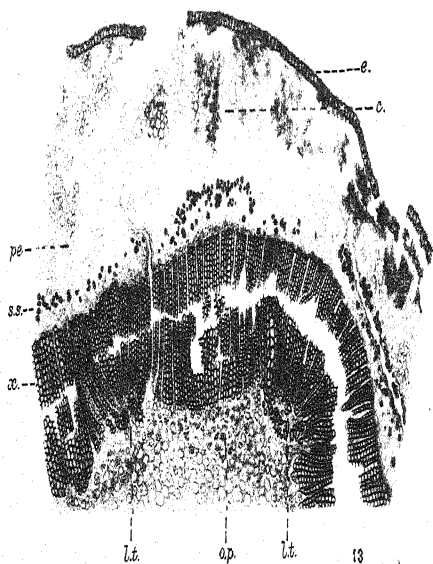


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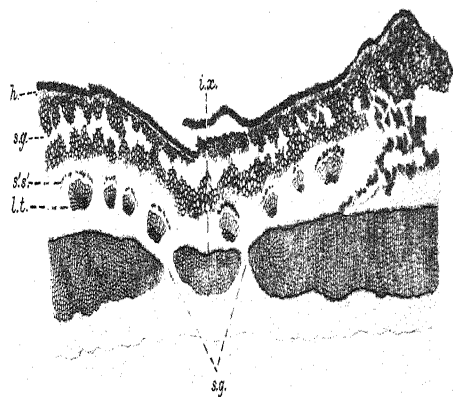


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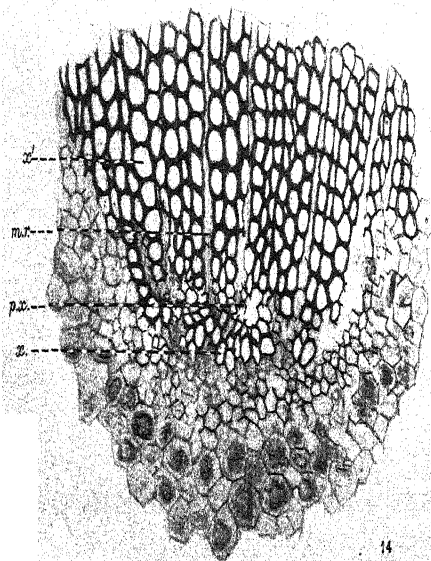




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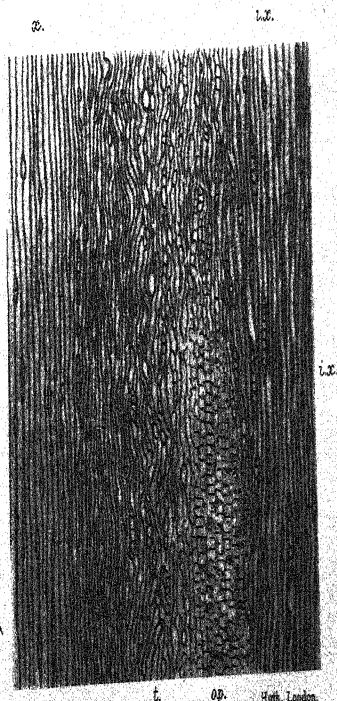
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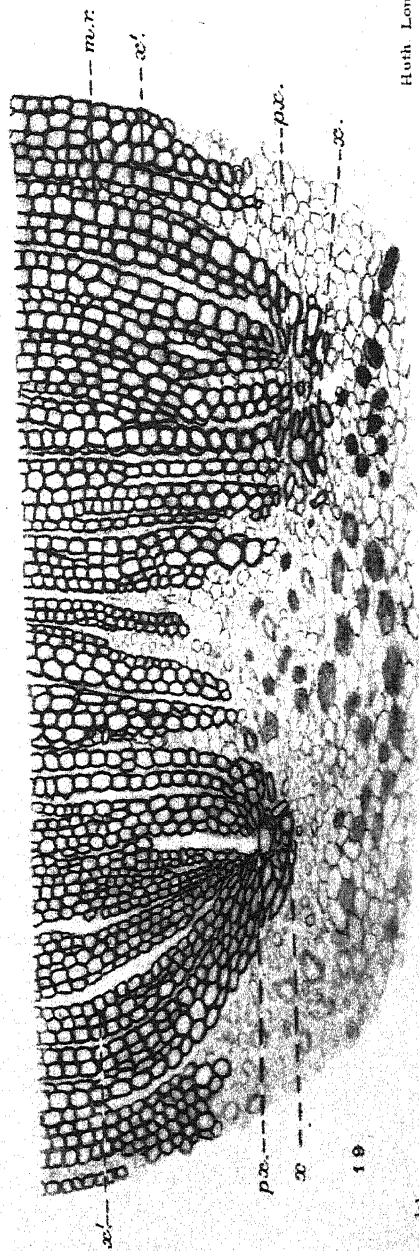
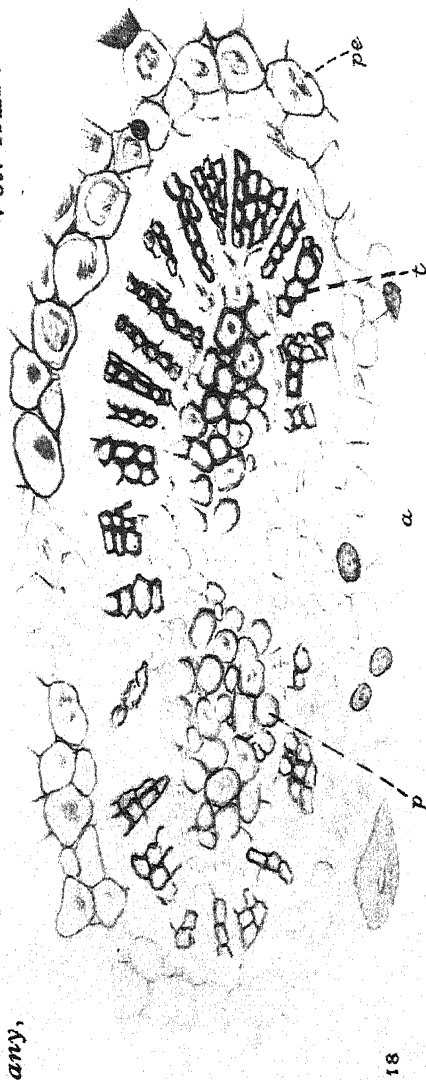
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Gwilliam & Maslen del.

MASLEN -- MESOXYLON & MESOXYLIDES.

Huth. London.

Microsporogenesis in *Taxus*.

BY

LILIAN E. HAWKER, B.Sc.

With twenty Figures in the Text.

THE reduction division of the microspore mother-cells in *Taxus* shows several features worthy of note since the actual method of reduction seems to differ considerably from that in any other conifer as yet described.

PROPHASES.

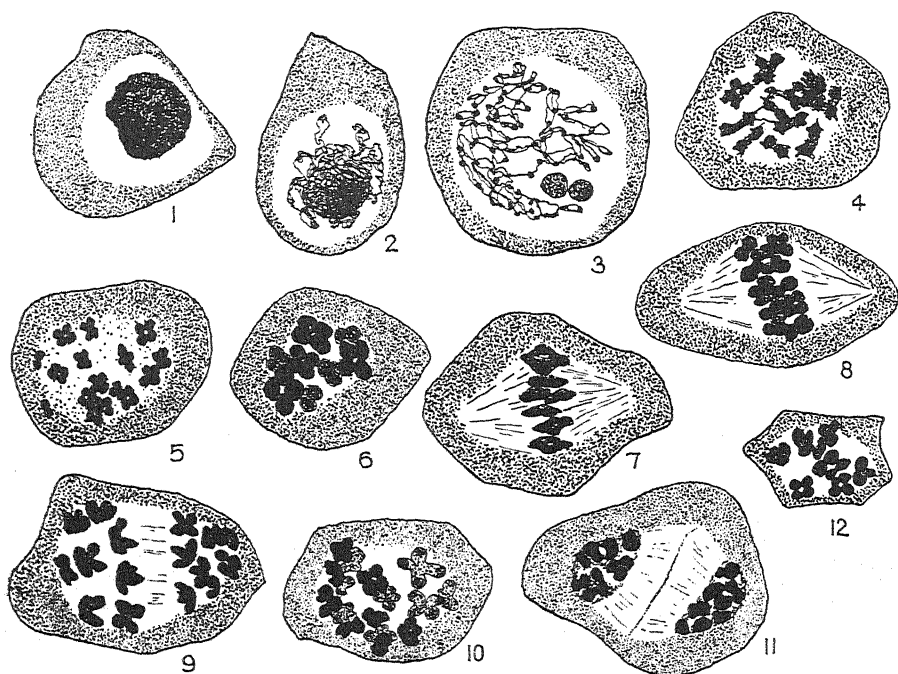
The nuclei of the microspore mother-cells pass into synapsis during October. The nuclear contents contract to less than half the size of the resting nucleus and are situated to one side of the nuclear cavity (Fig. 1). On recovery from synapsis the chromatin mass becomes less dense, and looped threads gradually extend into the cavity (Fig. 2), which eventually becomes filled with an evenly distributed, intertwined, chromatin thread. No definite indication of conjugation of portions of the spireme can be seen at this stage, nor do the threads appear to be of a double nature as described by Lewis (2) for *Pinus* and *Thuja*.

When the spireme thread has completely filled the nuclear cavity the chromosomes begin to be marked out, and appear as irregular, dark-staining masses connected by delicate threads, the position of which is indicated for some time by pointed projections from the chromosomes, giving the latter a very irregular appearance (Fig. 4). The chromosomes do not show any constant arrangement, although in some cases there are indications of the formation of typical chromosome tetrads, and in others there appears to be a more or less definite pairing of chromosome masses.

METAPHASE AND ANAPHASE.

The achromatic figure of the heterotype division is formed about the end of the first week in November. By examination of whole living cells, the haploid number of chromosomes is seen to be eight and the diploid sixteen, as already shown by Strasburger (4). It is in the remarkable behaviour of the chromosomes during metaphase that *Taxus* differs from all other conifers for which microsporogenesis has been described. When

the mother-cell is sectioned in the plane of the equatorial plate, the chromosomes are seen to be marked out into tetrads, each tetrad apparently consisting of four rounded masses in close contact. The cells appear to be of two distinct kinds, some with sixteen small chromosome tetrads in one



FIGS. 1-12. 1. Microspore mother-cell in synapsis. 2. Recovery from synapsis, looped threads extending into the nuclear cavity. 3. Spireme nearly filling cavity. 4. Formation of chromosomes during prophase of first division. 5. Polar view of chromosomes arranged on spindle; sixteen small chromosome tetrads. 6. Similar view of a cell with eight large chromosome tetrads. 7. Equatorial view of a cell similar to the preceding. 8. Chromosomes beginning to separate. 9. Late anaphase, showing chromosomes consisting of four short rods; equatorial view. 10. Polar view of the same stage as the preceding. 11. Early formation of daughter nuclei and transverse wall. 12. A polar view similar to the preceding.

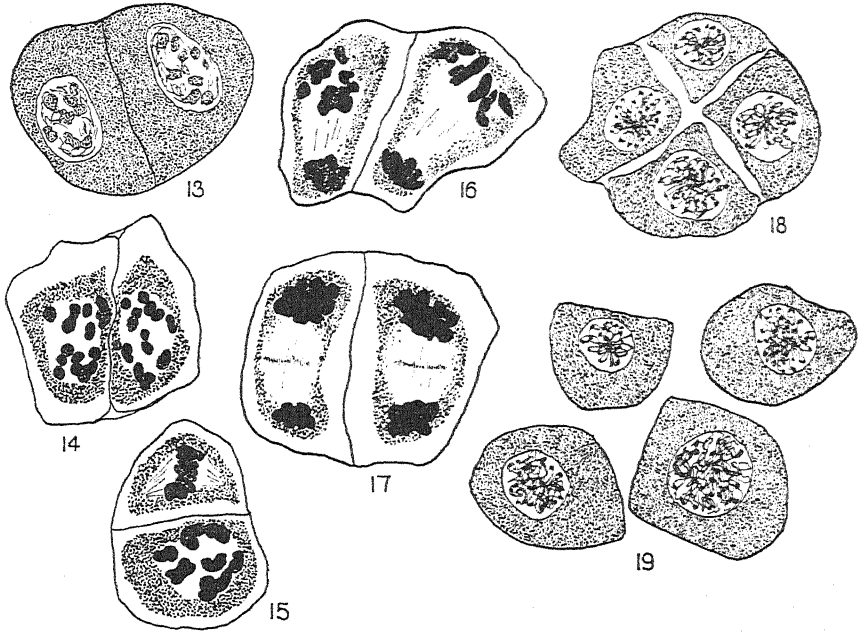
Figs. 1-12 were all drawn with the camera lucida, and reduced in reproduction to a magnification of $\times 2,000$.

plane and others with eight larger ones. This difference in size is proved to be a real one by the camera lucida drawings to the same scale reproduced in Figs. 5 and 6.

When the cells with sixteen small tetrads were closely examined, it was shown by careful focusing that the four component masses converged in one direction but not in the other, whilst the four parts of the larger tetrads converged in both directions. Secondly, when the plane of the section is perpendicular to the equatorial plate, the larger chromosome tetrads present the appearance of four slightly bent rods in contact with one another at both ends (Figs. 7 and 20). Unfortunately, in the cells with sixteen small

tetrads, the latter are too closely crowded on the spindle for any clear view to be obtained.

In all cases, during anaphase, the separation of daughter chromosomes gives the impression of whole tetrads passing to the poles, but a closer



FIGS. 13-19. 13. End of the first division, showing two daughter nuclei with distinct nuclear membranes and chromosome masses still distinct. 14. Metaphase of second division; polar view. 15. Similar stage with the two spindles perpendicular to one another. 16. Late anaphase of second division. 17. Wall formation at the end of the second division. 18. Four microspores within the mother-cell wall. 19. Microspores still arranged in a tetrad, but free from the mother-cell wall.

Figs. 13-19 were all drawn with the camera lucida, and reduced in reproduction to a magnification of $\times 2,000$.

examination reveals the fact that each separating chromosome mass consists of four short, thick, rods in contact at one end. However, the reduction division is brought about in the two types of cell the final result is the same in all cases, as might be expected (Figs. 8, 9, and 10). The appearance of the tetrads when seen in the plane of the equatorial plate and the tetrad-like appearance of the separating daughter chromosomes might be due merely to a longitudinal splitting of crescent-shaped chromosomes, as described for *Lilium canadense* by Allen (1), but this could not explain the distinct difference in size and number of chromosome tetrads at metaphase.

One possible method of reduction which would account for the difference in size and number of chromosomes, and at the same time give them the form observed during metaphase and anaphase, seems to be by a transverse split of all the component masses of the chromosome tetrads. In this

case the difference in size and number would be due to a difference in the time at which the transverse split takes place. In the case of the smaller tetrads, splitting presumably would occur before the chromosomes become arranged on the spindle, whilst in the case of the larger it would be delayed until the chromosomes are just about to separate.

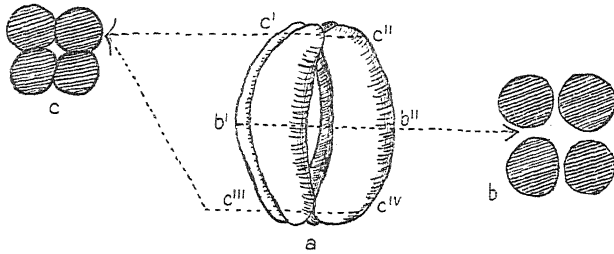


FIG. 20. Diagram of chromosome tetrad during metaphase of first division. (a) chromosome tetrad as seen in equatorial view; (b) the same in polar view when focused at plane $b^I b^{II}$ of (a); (c) the same when focused at planes $c^I c^{II}$ or $c^{III} c^{IV}$ of (a).

A more probable method of reduction would be for two of the component masses of each chromosome tetrad, i. e. a whole chromosome split longitudinally, to pass to each pole. The appearance of the chromosomes during anaphase as described above would then be due to the lagging behind of the four free ends of the two rods composing the chromosome, during their passage through the cytoplasm. The apparent difference in the size and number of the chromosome tetrads would be due to a difference in the time of separation of the paired chromosomes. Chromosomes of a somewhat similar appearance are figured by Lopriore (3) for *Araucaria Bidwillii*, but there is no evidence of any difference in their size.

TELOPHASE.

The daughter nuclei never attain the appearance of typical resting nuclei, since the chromosome masses remain visible, often as fairly distinct 'tetrads' which stain less strongly than during metaphase, and are connected by a loose network of nuclear material (Figs. 11, 12, and 13). A nuclear membrane is formed but soon disappears again.

SECOND DIVISION.

The second or homotype division takes place very rapidly and shows no features of particular interest (Figs. 14, 15, 16, and 17). The appearance of the young microspores is somewhat similar to that of the original mother-cells and they become free from the mother-cell wall at an early stage (Figs. 18 and 19). The two spindles of the second division may lie parallel or at right angles to each other so that the four microspores may or may not be in the same plane.

DURATION OF DIVISION.

An attempt was made to watch the actual divisions by pressing out living mother-cells into a drop of tap water or sugar solution. By this method certain stages of the division were successfully followed in living cells. The mother-cells were in synopsis for several weeks. In 1928 the cells were found dividing on any date between Nov. 9 and 19. The division was not simultaneous, all stages up to the formation of spore tetrads being observed in the same cone, although cells in the same loculus were usually in the same stage. The metaphase and anaphase of the first division took from two to three hours, as nearly as could be estimated. The whole of the second division was a rapid process, but attempts to follow it exactly in the living cell were less successful than in the case of the first.

Thanks are due to Mr. W. T. Saxton for advice and criticism.

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On the Cultural Behaviour of *Sphaeropsis malorum*, Pk.

BY

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AND

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With Plate XXIX and one Figure in the Text.

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I. INTRODUCTORY.

IN an earlier paper by one of us (Mohendra, 5) an account was given of the changes undergone in culture by four fungi, viz. *Neocosmospora vasinfecta*, *Alternaria tenuis*, and two species of *Phoma*. A fifth fungus,

Sphaeropsis malorum, was included in the investigation, but the behaviour shown in this case proved to be somewhat more complex, so much so that publication of the results was held over until further confirmation had been obtained. An extensive re-examination of the problem has now been carried out, the results of which substantially confirm, and in certain respects amplify, the earlier findings.

II. EXPERIMENTAL.

(a) *Method.*

The method of culture adopted in the first instance was the same as that used for the other four fungi already mentioned. This has been fully described in the earlier paper (pp. 866-9), and, briefly, was as follows. Parallel series of recultures were made on a variety of media, the inocula consisting of spores only, or young mycelium only, or old mycelium only. The object was to determine whether the fungus, after a series of such recultures, showed any tendency to vary in accordance with the nature of the inoculum used—whether, for example, the cultures carried on by spores tended to become more actively sporing, or whether those carried on from mycelium tended to become sterile.

It may be recalled here that the results already published showed that there was no such tendency to divergence, and that if care was taken to avoid saltant strains, which appeared with greater or less frequency in the different cases, each fungus remained unchanged in successive recultures, no matter what kind of inoculum was used.

As will be shown later, the peculiar feature of the fungus *Sphaeropsis malorum* is the occurrence of more than one kind of spore within the pycnidium. The experimental evidence bearing on this point will occupy the bulk of the present paper. The routine cultural experiment, on the lines of the earlier paper, need only be described in so far as it is necessary to understand how different types of cultures arose in the first instance.

(b) *Routine Cultural Experiment.*

The fungus, which was isolated from apple fruits obtained at Wye College, Kent, had already been kept in culture in the laboratory for a few generations. A single hyphal tip was cut out in the usual way, and this gave, in due course, the parent from which all subsequent recultures were derived.

Cultures were set up on three media—Brown's agar, Richards' agar, and prune juice agar, the three methods of inoculation described above being used in each case. In the very first generation of cultures on Brown's agar a marked difference was noticeable between the ones derived from mycelial inocula on the one hand and those derived from spores. The

former (see Pl. XXIX, Fig. 1) were whitish-grey in colour, with only a few pycnidia, which were large ($800-1,000 \mu \times 750-900 \mu$), and were either sterile or contained few spores. The latter (see Pl. XXIX, Fig. 2) were dark grey, and showed numerous pycnidia, which were small ($330-480 \mu \times 210-300 \mu$), and contained an abundance of spores.

It may be added here that while the feebly sporing type of culture is almost sterile when grown on Brown's agar, it sporulates quite freely on both the other media. Pl. XXIX, Fig. 3, illustrates the appearance shown on prune juice agar. The large pycnidia near the centre of the colony are fertile, while the great majority of the smaller structures towards the margin remain sterile, and are apparently abortive pycnidia. Under the same conditions the strongly sporing type of culture sporulates intensely (Pl. XXIX, Fig. 4), the numerous small pycnidia being all fertile.

It is interesting to note also that the difference in intensity of sporulation shown in culture is equally well marked when the fungus is growing on living apple fruit. Both types of cultures seem to be equally active in parasitism. The number of pycnidia per unit area of surface of the parasitized fruit is about three times as great in the one case as in the other.

The corresponding cultures on Richards' and prune agars behaved normally—that is, on each medium the same type of growth resulted with all three methods of inoculation. After three generations all these cultures were compared side by side by transferring them to Brown's agar, when it was found that they were all of the same type and indistinguishable from the poorly sporing cultures on Brown's agar.

The process of reculture by the method outlined above was continued over eight generations, a total of 165 transfers being made. During this time the vigorously sporing culture behaved in the manner which will be described below. The feebly sporing cultures remained steady, apart from a sudden change in one case (mycelial culture on Richards' solution in the eighth generation). This new strain sporulated more freely than its parent, but behaved similarly to the latter when tested by the plating-out method described in the following section.

(c) *Plating-out Experiments.*

During the course of the routine cultural work described above many samples of spores were examined microscopically in order to ensure the absence of pieces of old mycelium. It was then observed that the spores showed differences among themselves. Some were deep brown in colour, others were more or less hyaline, some were elliptical, others more egg-shaped, and others again almost circular. Such variations may occur among the spores from a single pycnidium, as is illustrated in Pl. XXIX, Fig. 5.

In order to determine whether the spores obtained from a given culture

of the fungus produced the same type of growth, a series of platings were made on Brown's agar. In the earlier tests a mixture of spores from different pycnidia was used, but at a later stage care was taken that all the spores originated from a single pycnidium. The procedure which was finally adopted was as follows.

A single pycnidium was picked up and placed in sterile water on a slide. The surrounding mycelium was teased out, and removed by transferring the pycnidium to fresh drops of water. When microscopic examination showed that all pieces of mycelium had been removed, the pycnidium was crushed between sterile cover-slips. The pycnidial case was then picked out, and the cover-slips bearing the suspension of spores dropped into a cool but still liquid medium. For each pycnidium 500 c.c. of medium poured into twenty $4\frac{1}{2}$ in. diameter Petri dishes were normally used. The large number of plates was necessary to enable a reasonable estimate to be made of the number and kinds of daughter colonies. A further particular reason will appear later.

The cultures from which pycnidia were taken were grown generally on Brown's agar. In the case of feebly sporing strains, this medium was unsuitable, inasmuch as the pycnidia were generally sterile or contained too few spores for the needs of the experiments. Recourse was had to prune juice agar, which gives greater sporulation. Subsidiary experiments also showed that sporulation falls off rapidly at temperatures below 20°C . (Opt. 25°), and that the presence of light strongly favours spore formation. The cultures intended for the production of spores were therefore kept in diffuse light in the laboratory, and in cold weather were placed near a radiator.

When spores derived from the heavily sporing type of culture illustrated in Pl. XXIX, Fig. 2, were plated out on Brown's agar colonies of two main types developed. These will be referred to as black and white. Two such dilution plates are illustrated in Pl. XXIX, Figs. 6 and 7. In addition to the difference in colour, the two types have a different habit of growth, the black one having sparse aerial mycelium with a tendency towards the non-staling habit of growth, whereas the white type has abundant heaped-up aerial mycelium with distinctly staling tendencies. Thus it happens that when the plates are photographed by transmitted light the differences may not be clearly shown, the greater depth of mycelium of the white type compensating for the darkness of the other. This is illustrated in Pl. XXIX, Fig. 11. Contrary to appearances, the black culture is actually the lower one.

An additional feature, to which attention will be drawn later, is that the black colonies appear later than the white ones, and it is for this reason especially that overcrowding of the colonies on the dilution plates is to be avoided.

It was thought at first that the occurrence of black and white colonies

in the plating experiments might be associated with the mixture of dark coloured and hyaline spores in the inocula. This, however, proved not to be the case. To quote a particular experiment, six dark-coloured and six hyaline spores were isolated by Keitt's method (4) and placed in separate Petri dishes. The resultant colonies were all of the white type. The colour of the spore, therefore, is of no apparent significance in the present connexion, and it is probable that colour is merely an index of the maturity of the spore.

It was mentioned above that colonies of the black type are the last to appear in the dilution plates. The view might, therefore, be put forward that they are black on this account, viz. that blackness is the result of a metabolic influence arising from the more advanced white colonies. The evidence against this view is overwhelming. Thus strains have been obtained in which the daughter colonies are all-black or all-white, independently of the number of daughter colonies on the plate. A direct proof was as follows.

Dilution plates were made from a culture known to give the two kinds of daughter colonies. Some of these plates were allowed to develop in the usual way, after which a count was made. In others each colony was picked out as soon as it was visible to the naked eye and grown singly in a Petri dish. It was then found that the percentage of black to white colonies was much the same in the two cases.

Parallel cultures of the strongly sporing strain set up on Richards' agar and prune juice agar gave rise to dilution plates essentially similar to those just described.

When, however, spores were taken from the feebly sporing strain illustrated in Pl. XXIX, Fig. 3 (culture on prune juice agar) the dilution plates contained only the white type of colony. Such a plate is illustrated in Pl. XXIX, Fig. 8.¹ All the poorly sporing cultures obtained in the course of the routine cultural experiments gave this result.

A study of the white colonies shown in Pl. XXIX, Figs. 6 and 8, suggests that they are not all similar. Thus some bear more pycnidia than others, one (v. Fig. 8) has a distinct pycnidial zone at the margin, some colonies are much smaller than others, and so on. Attempts have been made to carry on these different types of colony, but they behave with considerable irregularity. They always remain of the white type, and some cultures sporulate more freely than others, but it is not certain how far they remain steady in other respects.

In the preceding section it was stated that successive generations of recultures were set up, the strain which gives mixed black and white daughter colonies in the dilution plates being carried on by mass transfers

¹ In the photograph the small strongly-staked colonies shown near the upper margin appear dark, but this effect is due merely to the dense heaped-up mycelium.

of the spores. The spores of successive generations were then examined in due course by the poured plate method. These tests were begun at the fourth generation and extended to the ninth, a period of about eight months. Approximate counts were made of the numbers of black and white daughter colonies in the dilution plates, and it was found that the ratio of black to white diminished in successive generations. This result is illustrated in Table I.

TABLE I.

Generation of Culture.	Total No. of Colonies.	Black.	White.	Percentage of Black.
4th	53	22	31	41
5th	102	28	74	27·5
6th	107	13	94	12
7th	159	7	152	4·4
8th	93	0	93	0
9th	48	0	48	0
„	100	0	100	0

Thus in about four generations of recultures by mass transference of spores the percentage of black daughter colonies in the dilution plates fell from about 40 per cent. to 0 per cent.

A more extended investigation along the same lines, involving larger numbers of daughter colonies and using spores from single pycnidia, was then carried out. The initial culture was one which gave about 40 to 50 per cent. of black colonies on the dilution plates. The data for six pycnidia chosen at random are given in Table II.

TABLE II.

Pycnidia.	Black.	White.	Total.	% Black.
1	101	104	205	49
2	17	16	33	51·5
3	50	106	156	32
4	18	25	43	42
5	73	93	166	44
6	118	151	269	44
Total	377	495	872	Av. = 43·2

The first subculture (on Brown's agar), derived by mass transference of spores from a single pycnidium of the above culture, gave the results shown in Table III.

TABLE III.

Pycnidium.	Black.	White.	Total.	% Black.
1	25	26	51	49
2	75	102	177	44
3	3	54	57	5
4	1	15	16	6
5	0	14	14	0
6	97	125	222	44
Total	201	336	537	37·4

The next generation of subcultures gave the results shown in Table IV.

TABLE IV.

Pycnidium.	Black.	White.	Total.	% Black.
1	0	14	14	0
2	1	2	3	33
3	1	15	16	6
4	5	25	30	17
5	6	13	19	31.5
6	10	25	35	28.5
7	0	2	2	0
8	7	18	25	28
9	10	11	21	48
10	4	61	65	6
Total	44	186	230	19.1

The next subculture formed a fair number of pycnidia, but nearly all were sterile. Only a few spores were obtained, even after prolonged search, and these gave white daughter colonies. A parallel series of cultures on prune juice agar was, however, available. The spores from eleven pycnidia of the corresponding culture on prune juice agar were plated out, and gave no black daughter colonies out of a total of 502.

It is noticeable from the above tables, especially from Tables III and IV, that there is considerable diversity among the different pycnidia of the same culture, both as regards the number of spores contained in them and the percentage of black to white daughter colonies obtained on plating. Nevertheless, when one considers the total figures, a progressive diminution of the number of black daughter colonies in the course of successive recultures is plainly shown.

At the conclusion of the tests just described the four generations of cultures were inoculated side by side in Petri dishes of Brown's agar. When these cultures had developed, a distinct gradation in colour was shown, from dark in the oldest to white in the youngest. A progressive diminution in sporulating capacity was likewise shown.

The gradual change from a dark coloured to a white culture, with simultaneous loss of the black daughter colonies on plating, has been repeatedly confirmed with cultures on the three artificial media chiefly used in this investigation. From more limited observations it would also appear that the same tendency, in perhaps a more marked form, is shown when the cultures are maintained on living apple fruit. Thus inoculations were made on apples from cultures which gave 64 per cent. of black daughter colonies on plating. A similar examination of the spores from three pycnidia produced on the infected apples gave only 25 per cent. of black colonies.

(d) *Further Examination of Black and White Daughter Colonies obtained by the Dilution Method.*

Single colonies were isolated from the dilution plates and grown until pycnidia had developed. Spores from these were then plated out in the usual way (on Brown's agar) and the nature of the resulting daughter colonies observed.

The white colonies in the great majority of cases showed poor sporulation of the type illustrated in Pl. XXIX, Fig. 1, and gave only white daughter colonies when their spores were plated out. In some cases, however, a certain percentage of black colonies appeared in the dilution plates. The white cultures which thus gave a number of black daughter colonies on dilution were not exactly white in appearance, but would be better described as grey.

The black colonies, which showed good sporulation, gave rise to a mixture of black and white daughter colonies when their spores were plated out, and the percentage of the former was higher than was the case with the parent culture. Thus a particular culture gives, say, an equal admixture of black and white daughter colonies. When spores from a black daughter colony are similarly plated out the percentage of blacks may be as high as 100 per cent. The great majority give a certain degree of admixture with white, as illustrated in Pl. XXIX, Fig. 9, where there are five black daughter colonies and only one white. A number of *all-black* cultures have been isolated, these showing no white daughter colonies in the dilution plates (see Pl. XXIX, Fig. 10).

The general position may be summed up as follows. The colonies which arise in the dilution plates are not simply black or white, but at least four types can be distinguished :

- (i) all-black, which give 100 per cent. black daughter colonies when their spores are plated out ;
- (ii) black, which give a mixture of black and white on plating, the former being in the majority ;
- (iii) grey, which give daughter colonies as in (ii), but with a majority of whites ;
- (iv) white, which give only white daughter colonies in poured plates of the spores.

When a black culture is carried on through a number of generations by successive mass transfers of spores, the same gradual change takes place as already noted, viz. a progressive diminution in the percentage of black daughter colonies in the dilution plates. Thus a culture which gave at a certain date approximately four times as many black daughter colonies as white showed equal numbers of black and white three generations later.

It is thus seen that any culture of the mixed type tends to lose the black constituent by the ordinary routine process of transferring spores. The only method of preserving the black constituent is to make dilution plates at a time when black daughter colonies are still present, and to isolate one of the latter. The percentage of black daughter colonies will now be high, but the process of 'degradation' towards a pure white type begins again.

The all-black type of culture, on the other hand, shows greater stability. A number of these have been cultured in the usual way for several generations, and have been found to give only black daughter colonies when the spores are plated out. Thus, in one series of tests, twenty-four pycnidia from four generations of such a culture were examined. Altogether 1,060 daughter colonies were counted, and these were black in every case. An exception was noted in one case where a culture which had hitherto only given black daughter colonies gave approximately 20 per cent. of white colonies after being cultured on living apple fruit. Whether this was an exception to the apparent rule, or whether the particular culture was not in reality all black, was not ascertained.

(e) *Mixed Cultures.*

Two cultures, A and B, were used in these experiments. The former was an all-white form, which was almost sterile when grown on Brown's agar, though it sporulated fairly freely on prune agar. The culture B was a freely sporing all-black form.

Spores of both A and B were taken and placed in separate spots about one inch apart on a plate of Brown's agar. In course of time the two colonies met, and a line of pycnidia was formed along the junction (Pl. XXIX, Fig. 11). Three pycnidia on the black culture and away from the line of junction were tested by the dilution method, and gave 100 per cent. black daughter colonies (286 altogether). Though the white colony produced a fair number of pycnidia, practically all were sterile, and only twenty spores were obtained. These all gave white colonies as expected.

The pycnidia along the junction were at the same time tested. The majority of these were also sterile. The data for five pycnidia which contained spores are given in the following Table.

TABLE V.

Pycnidia.	Black.	White.	Total.	% Black.
1	5	17	22	23
2	0	16	16	0
3	4	30	34	12
4	0	19	19	0
5	1	5	6	17

Some of the pycnidia thus showed signs of intermixture of black and white types. The experiment was repeated several times, but while the line of pycnidia always appeared along the junction of the two cultures, it was difficult to obtain sufficient spores for purposes of the plating tests. A more satisfactory method was found to be as follows:

The two kinds of spores, instead of being placed some distance apart, were sown together in the same inoculum. At the same time inocula of each type of spore were placed on control plates. The culture arising from the mixed inoculum was intermediate in colour between the controls, as was to be expected. Fertile pycnidia were freely produced in the mixed culture, and when the spores were plated out in the usual way nearly all showed admixture of black and white types. Spores from pycnidia on the two control plates were examined in the same way, and found to give rise to either all-black or all-white daughter colonies. Table VI gives the data obtained from the examination of fifteen pycnidia produced on the mixed culture.

TABLE VI.

Pycnidia.	Black.	White.	Total.	% Black.
1	31	223	254	12
2	47	98	145	32
3	52	80	132	39
4	23	60	83	28
5	90	194	284	32
6	45	128	173	26
7	10	20	30	33
8	16	0	16	100
9	126	172	298	42
10	39	49	88	44
11	138	79	217	63
12	120	96	216	55
13	110	152	262	42
14	122	178	300	41
15	55	23	78	70

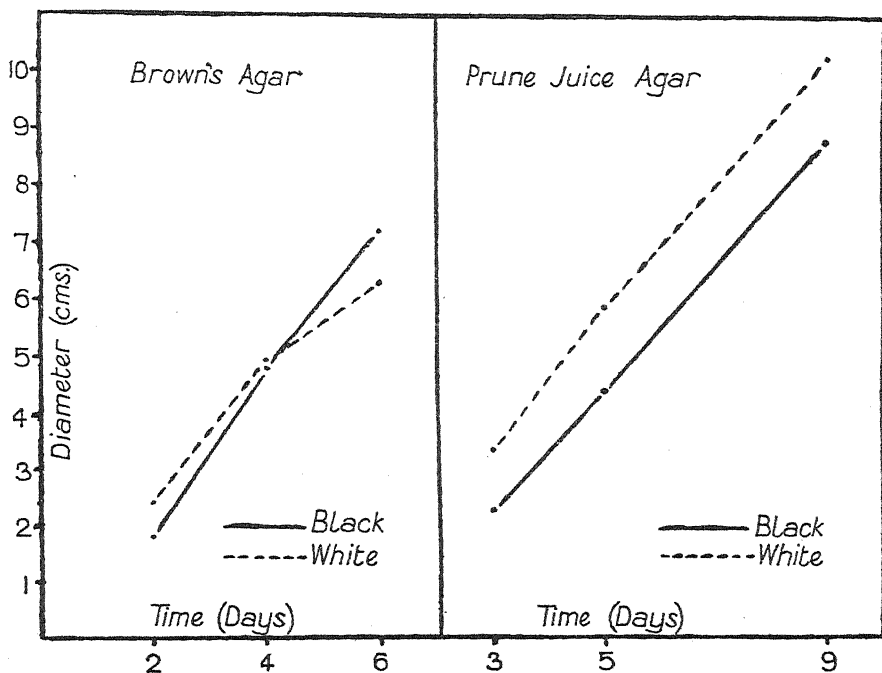
The above table shows clearly that the two types of culture have become thoroughly intermixed, even in the pycnidia.

(f) *Physiological Differences between Black and White Types of Culture.*

It was mentioned earlier (p. 544) that the black daughter colonies appeared somewhat later than the white ones in the dilution plates. This feature was examined more closely, as it seemed to afford an explanation in physiological terms of the peculiar cultural behaviour of the fungus. Two respects in which the black and white types of culture were found to differ were as regards:

1. Rate of spore germination and colony growth.
2. Viability of spores.

1. *Rate of spore germination and colony growth.* Spores which produce the black type of colony germinate more slowly than those which produce the white type. The difference is not marked when the spores are young,



TEXT-FIG. 1. Showing different rates of growth of black and white cultures on Brown's agar and prune juice agar.

but with older spores (i.e. spores from older cultures) it becomes very pronounced. The difference in rate of germination is seen equally well when the spores are suspended in water or in a nutrient. The following figures illustrate these points, the germination period being in each case sixteen hours :

Spores from young cultures (fifteen days old).

Average germ-tube in water.	white type.	85 μ
	black „	58 μ
Average germ-tube in nutrient.	white „	151 μ
	black „	75 μ

Spores from old cultures (two months old).

Average germ-tube in nutrient.	white type.	74 μ
	black „	1.3 μ

In the case of the two months old black culture only a small percentage of the spores had begun to germinate in sixteen hours. After forty hours about 50 per cent. had germinated, but the germ-tubes were still quite

short. By that time the germ-tubes of the spores from the white type of culture were so long that they were beyond the range of easy measurement under the microscope.

The same effect is seen in measurements of colony growth. Two typical samples are shown in Text-fig. 1. With prune juice agar as medium, both types of colony grow at much the same rate, so that the initial advantage of the white type is maintained throughout. On Brown's agar the white type of colony is likewise ahead at the beginning, but as it shows distinct staling reactions it is finally surpassed in diameter by the black type.

2. *Viability of spores.* The difference to be noted under this head is perhaps to be considered as the extreme case of that just described. As the cultures (and therefore the spores) become older, not merely do the spores of the black type lag behind the white as regards rate of germination, but a smaller percentage of them is capable of germination at all. It is probably for this reason that when plating tests are made at different times with spores from the same culture, the percentage of black colonies tends to diminish as the culture becomes older. Thus a certain culture gave 64.5 per cent. of black colonies (765 counts) when tested after two months' growth. The corresponding figure for the same culture three and a half months later was 43.8 per cent. (244 counts).

(g) *Comparison of Various Types of Culture from the General Systematic Point of View.*

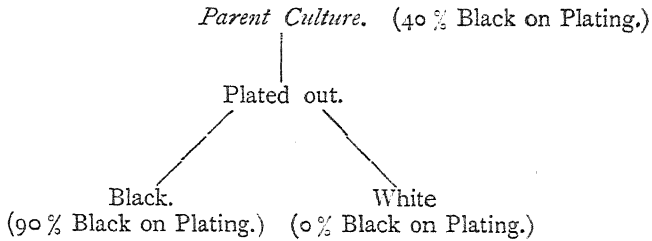
Several variant features have already been noted, e.g. size of pycnidia, mycelial colour, and intensity of sporulation. Variation in size and shape of spores from the same pycnidium has also been described, and this fact makes it difficult to determine whether there is any significant difference in this respect between the different types of culture. From time to time a certain percentage of two-celled spores was observed, and in one set of cultures this percentage rose to a high figure, so much so that a sample of spores would have been referred off-hand to the genus *Diplodia*. Occasional three-celled spores have also been observed.

The presence of two-celled spores in certain species of *Sphaeropsis* has been noted by a number of other workers, e.g. by De la Croix (2) and Diedicke (3) for *S. malorum* and by Archer (1) for other species. It is clear, therefore, that the line of demarcation between the two genera is not clearly marked.

III. DISCUSSION OF RESULTS.

The chief interest of the present study centres round that type of culture the spores of which are found on plating out to be of different kinds. For the sake of clearness, the behaviour of such a culture may

conveniently be indicated (in simplified form) as in the following scheme, the figures being illustrative:



When a mass transfer of spores of the parent culture is made one knows therefore that at least two different kinds of spore are present in the inoculum. The question therefore arises why, when a mass inoculation of spores is made from such a parent culture, the resulting culture is nevertheless on the whole similar to the parent. It is somewhat lighter in colour, and when its spores are plated out the ratio of black to white daughter colonies is found to be somewhat less than formerly. The essential feature is that the pycnidia of the subculture do not consist partly of some which give a high ratio of black to white daughter colonies on plating and of others which give white ones only.

The answer to this question is given by the experiments with artificially mixed cultures. Clear evidence was obtained that some kind of interaction had taken place, with the result that the pycnidia of the mixed culture were intermediate in character between those of the components. The simplest interpretation of that result is that the hyphae of both the components contribute to the development of the pycnidia, and therefore some of the spores inside are produced on one type of mycelium and others on the other. Hence the mixed spore population.

The whole phenomenon, however, cannot be explained by a simple mechanical mixing of two sets of mycelium, with resultant mixed type of pycnidium. For example, a mono-spore culture, unless it be of the all-black or all-white type, gives both black and white daughter colonies when its spores are plated out. If this feature arises from the presence of more than one kind of hyphae in the pycnidium there must have been previous segregation. The view suggested is, therefore, that there is abundant segregation of the type of bud variation in a growing culture of *Sphaeropsis malorum*. The saltant hyphal tips do not, as a rule, separate out as sectors,¹ but freely intermix, both in the vegetative part and in the pycnidia, where they give rise to the different kinds of spores. It is possible, of course, that fusions, resulting in hybrid mycelia, may take place, but it does not seem to be necessary to fall back on such an hypothesis.

¹ Saltation in the characteristic 'sector' form has only been noted twice in the course of this work.

The tendency for the percentage of black daughter colonies to become less in successive recultures is fully explained on the basis of the physiological differences shown to exist between different kinds of spores. The type which produces a black culture is slower in germinating, and therefore, in a mass inoculum, it would tend to be suppressed by the more rapidly germinating type of spore which produces a white culture. Hence the mixture would be white to a greater extent than was the parent; and so on progressively until in a few generations the black strain had been eliminated altogether.

S. malorum affords an instructive example of a fungus which *gradually* loses its sporulating capacity under cultural conditions, even when the precaution is taken to use sporal inocula at each transfer. The mechanism of this change is fairly clear, viz. it is the gradual replacement of a vigorously sporing black type of mycelium by a poorly sporing white type. The behaviour of this fungus does not appear to be essentially different from that of many others in which a sudden loss of sporulating capacity can be traced back to the inadvertent carrying over of an inoculum from a weakly sporing saltant area. The only difference is that in the latter case the saltant and parent areas are clearly separated, so that a portion of the one may be picked up to the exclusion of the other, and hence the observed change is sudden. In the case of *S. malorum* the saltant and parent mycelia are intimately mixed, even in the pycnidia, and it is only because of certain physiological differences that the one gradually replaces the other in the course of a number of generations.

IV. SUMMARY.

(1) When the spores from a single pycnidium of certain types¹ of culture of *S. malorum* are plated out they are found to give at least two kinds of daughter colonies, which are referred to as black and white. More particularly, four types of daughter colony can be distinguished, viz. all-black, black, grey, and white, according to the nature of the daughter colonies produced when their spores are in turn plated out.

(2) Starting from such a culture, when successive transfers are made using mass inoculations of spores, the percentage of dark colonies in the dilution plates becomes less and less, and finally a pure white type of culture is evolved. This change takes place on all the media tested, in some cases, e. g. apple fruit, perhaps more rapidly than on others.

(3) The progressive reduction, as shown on plating, in the number of black daughter colonies runs parallel to a gradual change from a dark to

¹ In the case of such cultures one cannot properly use the words 'race' or 'strain'. According to the view put forward in this paper, they are to be considered as mixed cultures, even when started from a single spore. Even if this interpretation is incorrect, the fact remains that they are unstable in culture, and therefore the term 'race' or 'strain' would be somewhat meaningless.

a light colour in the culture itself, and also to a gradual diminution of the capacity of the culture to produce spores.

(4) When an all-black and an all-white culture are grown intermingled the spores from individual pycnidia are of mixed type.

(5) Spores which give rise to the black type of colony are slower to germinate, and have a reduced viability as compared with spores of white type. These features explain the tendency for the black type of culture to change towards the white type in the course of successive generations got by mass transfer of spores.

(6) While saltation in the form of definite sectors has occurred very rarely with this fungus the phenomena described in the text are most readily interpreted on the assumption that segregation of the bud-variation type takes place freely in a growing culture, with resultant formation of a mixed type of pycnidium.

The subject of this paper was suggested to us by Professor W. Brown, to whom we wish to express our thanks for his continuous interest and help.

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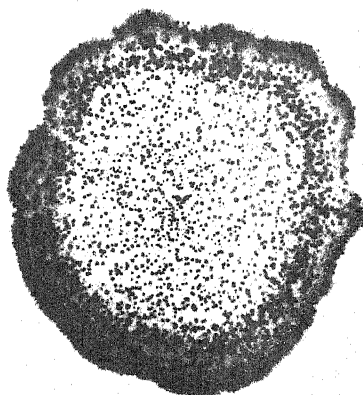
EXPLANATION OF PLATE XXIX.

Illustrating Dr. Mohendra and Dr. Mitra's paper On the Cultural Behaviour of
Sphaeropsis malorum, Pk.

- Fig. 1. White weakly sporing type of culture of *S. malorum* on Brown's agar. $\times 4/5$.
Fig. 2. Dark-coloured strongly sporing type of culture on same medium. $\times 4/5$.
Fig. 3. Same as in Fig. 1 but on prune juice agar. $\times 7/11$.
Fig. 4. Same as in Fig. 2 but on prune juice agar. $\times 7/11$.
Fig. 5. Microphotograph of spores from single pycnidium, illustrating variation in size, colour, and shape. $\times 300$.
Fig. 6. Dilution plate showing three black (one central, one on the right, and one at extreme left), and ten white colonies. $\times 7/11$.
Fig. 7. Dilution plate showing black and white colonies. These are not sufficiently separated to allow a count to be made. $\times 7/11$.
Fig. 8. Dilution plate showing white daughter colonies only. $\times 7/11$.
Fig. 9. Dilution plate showing five black colonies and one white one (extreme left). $\times 7/11$.
Fig. 10. Dilution plate showing black colonies only. $\times 7/11$.
Fig. 11. Black (lower) and white types of colonies growing side by side and forming a line of pycnidia along the junction. $\times \frac{1}{2}$.



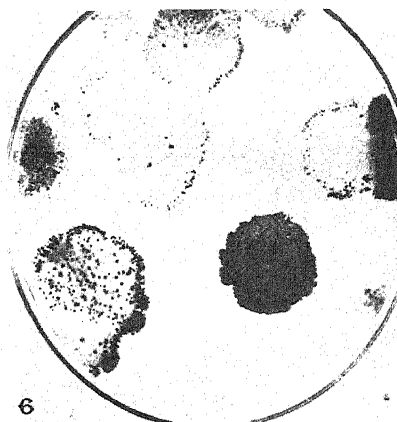
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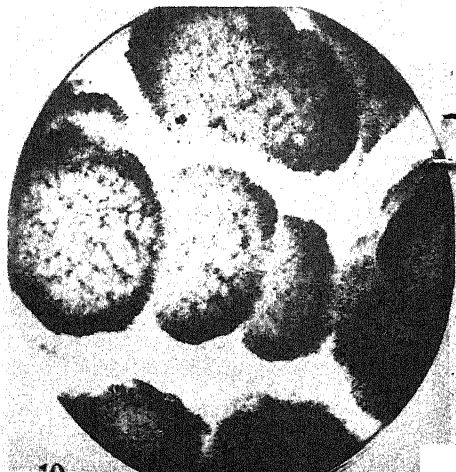
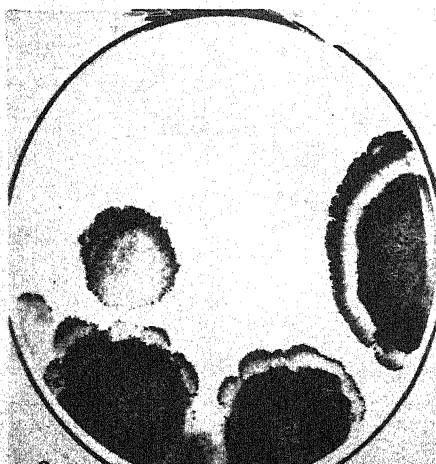
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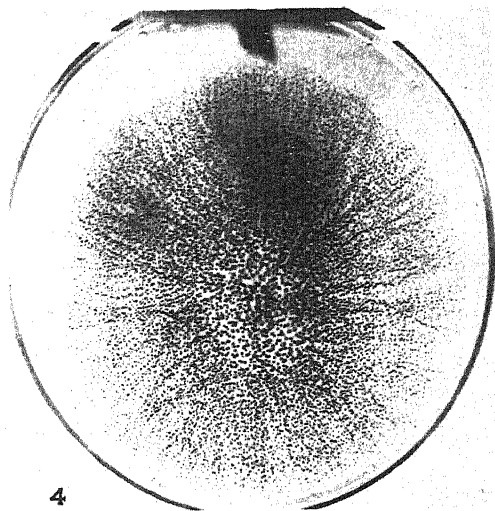
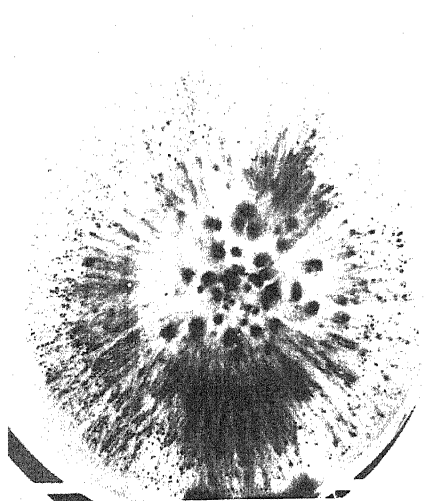
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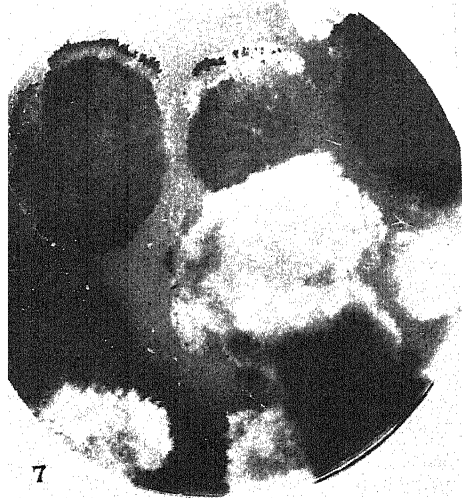
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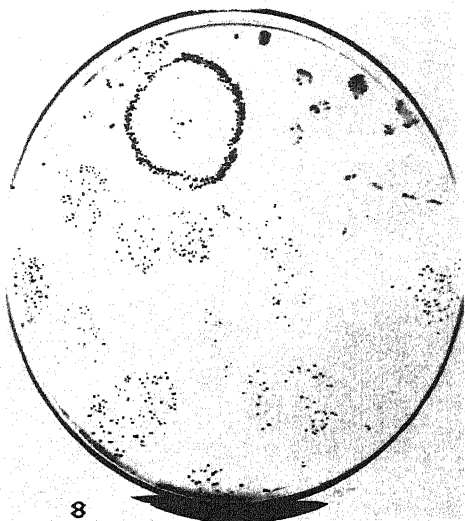
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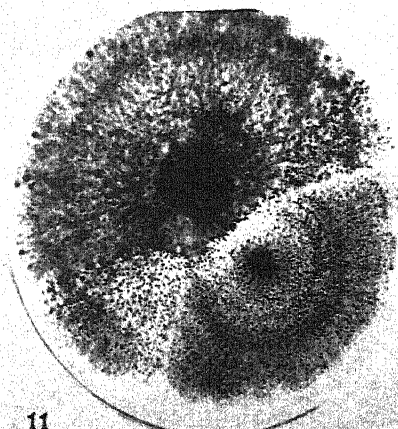
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11

Studies in the Physiology of Parasitism.

XII. On the Effect of One Organism in Reducing the Parasitic Activity of Another.

BY

R. SAHAI VASUDEVA, PH.D., D.I.C.

INTRODUCTORY.

IN the preceding number of this series (clxxiv, p. 469), the chief problem under consideration was the contrasting behaviour of *Monilia fructigena* and *Botrytis Allii* in relation to apple tissue. The former in a normal way is actively parasitic, the latter not at all. In attempting to bridge this gap, methods were devised for modifying the host-parasite relationship so that the normally non-parasitic fungus was able to attack. These have been described in the preceding paper. At the same time experiments were carried out to see whether conditions could be found in which the activity of the parasitic fungus could be reduced. An interesting method by which this can be effected is described in this paper. The results are very clearly marked, and appear to possess a considerable degree of generality. Though it is not obvious at the moment just what interpretation they will bear with regard to the problem of the physiology of parasitism, they are nevertheless cognate to the subject, and an account of them is therefore included in this series.

METHOD AND MATERIAL.

The general plan of experiment was to place three inocula on each one of a set of apples:

- (i) *B. Allii* alone.
- (ii) *M. fructigena* alone.
- (iii) Mixed inoculum of *B. Allii* and *M. fructigena*.

The inoculations were made in a standardized way by Granger and Horne's (3) method at more or less equidistant points round the 'equator' of each apple. The concentration of spores for each fungus was the same whether used separately or in the mixed inoculum.

It is known that considerable differences in resistance are shown by

apples, even of the same apparently uniform batch (Gregory and Horne (4), Harvey (5)). The factor of variable resistance could only be properly controlled by comparing the rates of attack on the same apple. Gregory and Horne (l. c.) have shown that the resistance to attack of different parts of an apple is sensibly uniform.

Experiments were as a rule carried out with batches of about ten to twelve apples, on each of which the three inocula indicated above were placed. After a suitable time (five or six days) the amount of rot produced by the various inocula was determined by weighing.

The earliest experiments were carried out with the pair of fungi already mentioned. Later other fungi were used, of which the following is a list :

Parasitic on Apple.

Botrytis cinerea.

Sphaeropsis malorum.

Penicillium sp.

Non-Parasitic on Apple.

Fusarium caeruleum.

F. fructigenum, strain B iii.

Cunninghamella elegans.

Helminthosporium Maydis.

Actinomyces tricolor.

Eidamia tuberculata.

Bacillus subtilis.

The variety of apple used throughout was Newtown.

EXPERIMENTAL RESULTS.

The results of a typical experiment are set out in Table I, the figures representing weights of rotted tissue in grammes.

TABLE I.

Apple.	<i>M. fructigena</i> .	<i>B. Allii</i> .	<i>M. f.</i> + <i>B. A.</i>
1	10.9	0.0	1.5
2	5.9	"	1.2
3	4.9	"	1.6
4	3.6	"	1.3
5	8.8	"	3.0
6	7.4	"	2.0
7	5.7	"	1.2
8	6.0	"	3.3
9	6.3	"	1.5
10	9.4	"	1.0
11	6.9	"	1.2
12	5.2	"	1.5
13	10.5	"	4.0
Av.	6.28	0.0	1.64

The statistical significance of the differences of attack recorded was evaluated by 'Student's Method' as described by Fisher (2). In this method a function t is calculated from the data, and according as this is

a larger or smaller number, the greater or smaller is the probability that the result recorded is significant. For a series of about twelve observations a value of t equal to 2.2 is on the margin of significance (the usual 20:1 standard being adopted). The value of t for the comparison:

M. fructigena against *M. fructigena* + *B. Allii*

as shown in the above Table is 8.9. This represents an enormously high probability (many thousands to one) that the attack is significantly greater when the active fungus is used alone.

The same type of experiment was repeated, using various pairs of the fungi given in the list above. The results from nine such experiments are given in Table II.

TABLE II.

No. of Apples.	Active Fungus.	Av. Amt. of Attack.	Mixed Inoc.	Av. Amt. of Attack.	t .
13	<i>M. fructigena</i>	6.3 grm.	<i>M.f.</i> + <i>B. Allii</i>	1.6 grm.	8.9
10	"	23.4 "	" + <i>F. caeruleum</i>	15.1 "	4.9
10	"	16.6 "	" + <i>F. fruct.</i> , B iii	13.1 "	2.2
10	"	12.7 "	" + <i>Cunn. elegans</i>	6.0 "	6.4
10	"	19.3 "	" + <i>Helm. Maydis</i>	16.6 "	3.1
9	"	17.7 "	" + <i>Act. tricolor</i>	12.1 "	2.9
9	<i>B. cinerea</i>	2.6 "	<i>B. cin.</i> + <i>B. Allii</i>	0.9 "	4.7
10	<i>Sph. malorum</i>	20.0 "	<i>Sph.</i> + " "	7.0 "	4.3
10	<i>Penicillium</i> sp.	9.2 "	<i>Pen.</i> + " "	7.8 "	4.1

All the comparisons shown above are clearly significant, except in the test with *F. fructigenum*. Here the result is of the same type but just barely significant.

In a parallel series of tests, the inoculum of the non-parasitic organism was introduced six days before the spores of the parasite were added. The exact procedure is perhaps best illustrated as follows:

June 1: Three cavities made in apple; one of these inoculated with saprophyte; all cavities closed up.

June 7: Drop of spore suspension of parasite added to cavity previously inoculated with saprophyte, another drop added to one of the uninoculated cavities, the third cavity being left as a control.

It was essential that all the cavities should be made at the same time, as a subsidiary experiment clearly showed that freshly made cavities were much more readily attacked than old ones.

The data of this series are given in Table III.

Comparison of Tables II and III shows that in the latter case the data are even more significant, the value of t being higher in the experiments of Table III than in the corresponding ones (carried out simultaneously) of Table II.

TABLE III.

No. of Apples.	Active Fungus.	Av. Amt. of Attack.	Mixed Inoc.	Av. Amt. of Attack.	<i>t.</i>
10	<i>M. fructigena</i>	26.3 grm.	<i>M.f.</i> + <i>B. Allii</i>	4.2 grm.	10.6
10	"	18.8 "	" + <i>F. caeruleum</i>	2.9 "	6.7
10	"	13.1 "	" + <i>F. fruct.</i> , B iii	1.0 "	9.9
10	"	13.4 "	" + <i>Helm. Maydis</i>	7.0 "	3.5
10	"	7.2 "	" + <i>Eidamia</i> sp.	2.0 "	4.4
10	"	13.5 "	" + <i>Cunn. elegans</i>	3.8 "	7.2
9	"	14.4 "	" + <i>Act. tricolor</i>	9.9 "	4.1
10	"	20.7 "	" + <i>B. subtilis</i>	14.0 "	3.6
10	<i>B. cinerea</i>	10.0 "	<i>B. cin.</i> + <i>B. Allii</i>	2.9 "	5.6
10	<i>Sph. malorum</i>	23.3 "	<i>Sph.</i> + " "	3.5 "	11.6

One thus reaches the conclusion that the addition of a saprophyte to the inoculum of a parasite diminishes the attacking power of the latter, at least in the case of apple tissue. This reduction of attack is still more marked if the saprophyte is present in the wound some days before the spores of the parasite are added.

The same kind of result is also obtained when, instead of the combination, parasite + saprophyte, one uses a mixed inoculum of two parasites. Thus in two experiments, in each of which twelve apples were used, the following average amounts of attack were obtained:

(1)	<i>M. fructigena</i> alone.		5.5 grm.
	<i>B. cinerea</i> "		0.5 "
	<i>M.f.</i> + <i>B. cinerea</i>		2.6 "
(2)	<i>M. fructigena</i> alone.		5.0 "
	<i>Penicillium</i> sp. "		0.4 "
	<i>M.f.</i> + <i>Penicillium</i> sp.		2.1 "

In both cases the mixed inoculum gave significantly less attack than did the more active of the two fungi ($t = 4.7$ and 4.2 respectively).

The results shown in Tables I and III might be taken as suggesting that something in the nature of immunization had taken place: viz. that through the action of the saprophytic fungus the tissues of the host had been rendered less susceptible to the action of the parasite. The fact, however, that quite a similar result is obtainable when the added fungus is also a parasite would discount this view. The probabilities are that the effect is simply one of the interference of one fungus with the growth of the other. The phenomenon appears to have some degree of generality, as is seen from the variety of fungi tested.

Similar effects have been reported by a number of other workers. Thus Porter (7) claims that the attack of wheat by *Helminthosporium* and of flax by *Fusarium lini* was reduced by the antagonistic action of a bacterium upon the fungus. In another paper (8) he records the observation that in

mixed culture the hyphae of one fungus may show a dissolving effect upon the hyphae of the other. Similarly Millard and Taylor (6) state that the presence of saprophytic species of *Actinomyces* markedly reduces the attack of the potato-scab organism. This effect they consider to be due to competition for food material and not to the setting up of unfavourable soil reactions.

On the other hand Fawcett (1) states that the attack of *Pythiacystis citrophthora* on citrus is increased in severity by adding a species of *Fusarium* to the inoculum.

A physiological analysis of the effect has only been attempted in the case of the two fungi *M. fructigena* and *B. Allii*. Whether the results obtained for these particular fungi are at all of general applicability is a question that further work must decide.

The same depression of the parasitic activity of *M. fructigena* can be obtained when the fungus *B. Allii* is replaced by its metabolic products. Two types of experiment were carried out in this connexion. In one a comparison was made between the rate of attack of *M. fructigena* spores sown in water and that in a watery extract of washed freshly germinated spores of *B. Allii*. The average amounts of rot produced (ten apples were used) were :

<i>M. fructigena</i> in water.	11.4 grm.
„ in watery extract of <i>B. Allii</i> spores.	7.6 „

The difference observed is definitely significant ($t = 2.5$).

More striking results were obtained in the second series when the following comparisons were made :

- (i) Spores of *M. fructigena* in apple extract of full strength.
- (ii) Spores of *M. fructigena* in the stale liquid derived from a flask culture of *B. Allii* in apple extract.
- (iii) Spores of *M. fructigena* in the stale liquid derived from a similar culture of *M. fructigena*.

The average amounts of rot produced in a series of comparative inoculation tests under the different conditions mentioned above are given in Table IV (twelve apples used in each experiment) :

TABLE IV.

Inoculum.	Av. Amt. of Rot.	t .
<i>M. fructigena</i> in fresh apple extract	10.6 grm. }	5.1
<i>M. f.</i> in 7 days' stale liquid from culture of <i>B. Allii</i>	8.8 „ }	
<i>M. f.</i> in fresh apple extract	23.0 „ }	16.9
<i>M. f.</i> in 50 days' stale liquid from culture of <i>B. Allii</i>	0.16 „ }	
<i>M. f.</i> in fresh apple extract	5.7 „ }	0.3
<i>M. f.</i> in 42 days' stale liquid from culture of <i>M. f.</i>	5.2 „ }	

It is clear that considerable reduction of parasitism occurs when spores of *M. fructigena* are sown in the stale liquid from a culture of *B. Allii*, and that the effect increases with the age of the culture of the latter fungus. The very small amount of rot produced by *M. fructigena* when sown in the stale liquid from a fifty days' old culture of *B. Allii* is very noteworthy. Out of the twelve apples thus inoculated, only three showed traces of attack after six days. On the other hand, no such reduction of parasitic activity is shown when spores of *M. fructigena* are sown in a solution containing its own staling products.

The reduced parasitic activity of *Monilia* spores when sown in the stale liquid from a culture of *B. Allii* is correlated with reduced capacity for germination and growth under these conditions. Cultures of *B. Allii* were set up in flasks on the following media—sterilized apple extract of full strength, potato extract, and Richards's solution. At intervals of ten days the liquid was filtered off from these cultures and tested with spores of *Monilia*. Table V gives the average length (μ) of germ-tubes of *Monilia* after twelve hours' growth at 20° C. in the various stale liquids.

TABLE V.

Medium.	Age of Stale Culture.			
	10 days.	20 days.	30 days.	50 days.
Stale Apple Extract	0	0	0	0
Stale A. Extract boiled	191	165	153	148
Stale A. Extract diluted to } 50 % with water	45	44	52	0
Stale A. Extract diluted to } 10 % with water	213	154	209	151
Stale Potato Extract	321	258	292	212
Stale Richards's Solution	147	146	278	246

It is clear that apple extract is the only one of the three media tested on which *B. Allii* rapidly produces staling substances which inhibit the germination of *Monilia* spores. That the effect of the stale apple extract is due to the presence of an inhibitory substance, and not to scarcity of nutrient, is shown by the results obtained with diluted stale extracts. The staling factor, furthermore, is removed by boiling. The pH of the fresh apple juice was 5.2, and even after fifty days' growth of *B. Allii* had only shifted to 5.4–5.6. The corresponding figures for potato extract were 6.4 and 7.4, and for Richards's solution 5.2 and 6.0. Thus the effects on germination of *Monilia* spores have no connexion with pH changes in the stale media.

While there is evidence that the fungus *B. Allii* when grown in apple extract rapidly produces substances which inhibit the growth of *M. fructigena*, the substances so formed do not materially affect the growth of *B. Allii* itself, as was pointed out in the preceding paper of this series. It is possible, therefore, to explain the reduction of parasitism shown by

Monilia in presence of *B. Allii* on the basis of staling effects. Whether this explanation applies to the other non-parasitic organisms tested in Tables II and III can only be determined by further work.

An alternative explanation of the repressive effect of *B. Allii* upon *M. fructigena* is that the former grows more rapidly than the latter, at least on the media tested. The media used in this connexion were potato agar, 50 per cent apple extract agar, and Brown's agar. When separate inocula of these two fungi were placed on plates of these media, it was seen that *B. Allii* was distinctly the more rapid grower. The effect was seen more convincingly when mixed inocula were tested. In such cases the colonies which grew out consisted almost entirely of *B. Allii*. In some plates the presence of *Monilia* could be detected in the neighbourhood of the mixed inoculum, in others no trace was discernible. The same result was obtained even when the spores of *Monilia* were present in large excess in the mixed inoculum.

To a certain extent the effects described in the preceding paragraph may be explained on the basis of staling phenomena, and thus the second explanation is hardly an alternative. That *B. Allii* appears to be intrinsically a more rapidly growing fungus than *M. fructigena* is almost certainly an accidental circumstance. The fact that a very slow-growing fungus like *Actinomyces* has a similar depressing effect on the parasitism of *Monilia* points to the same conclusion.

The author is greatly indebted to Professor W. Brown for valuable help and criticism.

SUMMARY.

1. The presence of *Botrytis Allii* in the inoculum of *Monilia fructigena* very markedly interferes with the vigour of parasitic attack by the latter. A similar effect was demonstrated for a number of other organisms.

2. The reduced parasitism shown by mixed inocula can probably be explained on the basis of staling phenomena and does not indicate tissue immunization.

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Notes on Cytoplasmic Structure in the Gymnosperms.

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With Plates XXX and XXXI and one Figure in the Text.

IN a series of recent studies the senior author of this paper has been attempting an analysis of the formed elements in the cytoplasm of plant-cells. The examination of the situation, both in differentiated cells and in meristematic tissue, has thus far been extended to representatives of the Bryophyta, Pteridophyta, and Angiospermae (2, 3, 4, 5, 6, 7). The study of this varied material has revealed not only a common plan of structural organization, but also a remarkable similarity in the morphology of the various elements involved. In these three large subdivisions of the plant-world, five kinds of structural components have been found to be of universal occurrence. These have been tentatively termed the plastidome, pseudochondriome, vacuome, osmiophilic platelets, and lipoidal droplets (4). The last mentioned probably stands in a somewhat different relation to the cytoplasmic organization than do the first four classes of materials. The lipoidal droplets presumably represent metabolic products of cellular activity which go and come like the fatty droplets in animal-cells. The other components probably have a much more intimate relation to the underlying, permanent organization of the cytoplasm, and are in some sense a part and parcel of living matter itself.

This plan of structure is apparently so widespread in plant-cells that it seemed of interest to extend the observations to gymnosperm material, in which as yet the matter has been rather incompletely studied. The present paper records the main features of our study of the cytoplasmic situation in the pine. The results here described are in no sense exhaustive, and the purpose of the paper is only to demonstrate that the formed elements of the cytoplasm in gymnosperms are exactly comparable to those

already found in the other higher plants. The material studied comprised the very young and entire seedling and the root-tip and growing-end of older seedlings in various stages of their early growth.

MATERIAL AND METHODS.

For this study the seeds of four species of the genus *Pinus* were used, viz. *edulis*, *sylvestris*, *Mughus*, and *austriaca*.¹ Of these, the species *edulis* received little attention, in part because the seeds failed to germinate readily, but chiefly because both primary root-tips and seedlings are very large. Experience in this laboratory indicates very strongly that large objects react unfavourably to those methods of osmic-fixation which are particularly important in cytoplasmic study. *P. sylvestris* also proved rather unfavourable on account of the technical difficulties introduced by its very abundant oil-droplets. The species *Mughus* and *austriaca* proved best adapted to the purposes of this study.

The primary root-tips were fixed when a few millimetres long, the seeds having been germinated in two different ways. One method of procedure was to soak the seeds for twenty-four hours in distilled water and then place them in moist chambers (in the dark) wet with distilled water.² Other seeds were planted in pots in sand,³ kept moist with tap-water, and left in the greenhouse at a moderate temperature for germination. The young seedlings were obtained (from seeds which had been a longer or shorter time in the moist chambers) by peeling off the seed-coat and splitting the endosperm-mass with a scalpel. The youngest seedlings, which are perfectly straight, are easily removed intact. In some cases older seedlings were removed in the same way after the root-tip (already through the seed-coat) had been cut off for fixation.

For purposes of study, root-tips which have grown to a length of a few millimetres are much superior to those still within the seed-coat. The other parts of the very young seedling, particularly the leaves, are also rather unsatisfactory. Our results are, therefore, drawn largely from study of young root-tips. In general, however, it is clear that the conditions in the hypocotyl and growing point of the seedling bear the same relation to those in the root-tip as has been observed generally in plants by Bowen (6) and other workers.

The primary root-tips of the pine possess certain rather striking

¹ The seeds were presented by the Stumpp and Walter Co., 30 Barclay Street, New York City, to whom our best thanks are due. The names given above for the species are those used in the trade. According to Bailey (1), in this country *Mughus* is more properly a variety of *Pinus Mugo* and similarly, *austriaca* is a variety of *Pinus nigra*. For our purposes it seemed most convenient to use merely the names of the varieties.

² See also Bowen (4), p. 694.

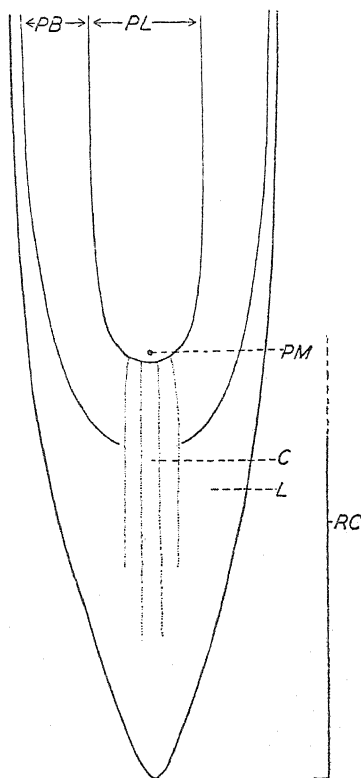
³ This method of germination is indicated by the letter 'S' in the fixation and staining formulae appended to the description of each figure in the Plates; cf. pp 585 and 586.

anatomical features, which in some respects differ from the typical arrangement in angiosperms. In order to locate more accurately the position of cells to be described and figured, the general topographical subdivisions of a pine root-tip are sketched in Text-fig. 1, together with the terminology which we have found convenient. The most characteristic feature of the root-tip is the presence of several columns of cells arranged in rather accurate lines running from the lower border of the promeristem toward the free tip of the root (*C*, in Text-fig. 1). The peculiar arrangement of the younger periblem zone is also to be noted. The promeristem region is concentrated at the tip of the plerome-cylinder. Our studies were confined to approximately that part of the root-tip included in Text-fig. 1.

The technical methods employed in this study were essentially the same as those already described by Bowen (4, 5). Two general lines of attack were followed: (1) that of osmic-impregnation; and (2) the methods used particularly by animal cytologists for the study of chondriosomes. The studies of the senior author, previously referred to, have demonstrated the value and uses of these methods as applied to plant-material.

Of the various methods of osmic-impregnation the one employed was that of Kolatchev.¹ Of the possible modifications of this method, we used only those in which osmication is carried out in 2 per cent. osmic acid; particularly the variation calling for an incubation at 40° C. for eight hours, before placing in the 35° incubator. Osmication-periods of five to seven days proved most generally successful.

By the Kolatchev method it was possible to blacken, on occasion, the plastidome, pseudochondriosomes, and osmiophilic platelets, very rarely the vacuoles—either alone or in various combinations. The extraordinary variability and wide range of the results obtained by the Kolatchev method on plant material has already been emphasized and discussed by



TEXT-FIG. 1. Diagrammatic longitudinal section of a main root-tip of *Pinus*, to show the approximate arrangement and extent of the regions examined in this study. *PB.*, periblem; *PL.*, plerome; *PM.*, promeristem; *RC.*, root-cap; *C.*, central columns of cells highly characteristic of the pine root-cap; *L.*, lateral region of root-cap.

¹ For complete details see Bowen (4, 8).

Bowen (4, 8, &c.). It will suffice for the present purpose to state that all sorts of results were obtained on the pine root-tips, sometimes touching extensive areas of cells, sometimes only single cells. In the case of other parts of seedlings the results of osmic impregnation were very much less useful, and in the special case of the promeristem in root-tips still within the seed-coat, rather uniformly unsuccessful. It may be particularly noted that in cells of the pine, blackening of the vacuome was very rare, and was never obtained in most types of cells; and further, that intense blackening of the pseudochondriome was rather frequent—in which respect the results differ markedly from those obtained in many other species of plants (cf. Bowen, 4, 5, 6). Further details of the behaviour of the Kolatchev method will be noted in the accompanying descriptive section.

One very striking feature of the gymnosperm-cells requires a word of special mention. This is the enormous quantity of lipoidal or oily material which is characteristic of all the cells in both seedling and growing root-tip. This material is distributed in the form of small droplets which, after osmic impregnation, are almost never actually blackened, but acquire instead a dark brownish tone, which makes the droplets very easy to identify. These droplets are so abundant in pine cells that they seriously interfere with osmic impregnation, and often ruin the results completely. But it is a matter of unusual interest that in root-tips from seeds germinated in sand, as noted above, the oil-droplets are often nearly or quite invisible, and the resulting preparations look much like those customarily obtained with angiosperm root-tips. Evidently the slightly different conditions of growth completely change the reaction of the fat-droplets to osmic methods. Bowen (4) has also noted a marked difference in the results of osmicating the root-tips of *Vicia Faba*, according as the seeds have been germinated in moist chambers or in soil.

Of the second type of technical methods, we have made use of the Mottier-Benda and Champy-Kull methods following the outlines as noted by Bowen (5) in the case of angiosperms. In order to eliminate certain difficulties introduced by the abundance of oil-droplets, bleaching either with hydrogen peroxide or by the familiar permanganate-oxalic method was usually practised, although the Benda stain can sometimes be successfully applied directly without any staining of oil-droplets. Nevertheless, bleaching often does not lead to a satisfactory end-result, especially in the case of very young seedlings. The handling of the Kull stain requires considerable trial in order to strike successful combinations of the various dyes involved. A count of our preparations from which drawings were made shows that the Benda method was most successfully applied to root-tips grown in sand; while with the Kull stain the method of germination seemed of less consequence.

The staining methods of Benda and Kull have yielded results com-

parable in a general way to those obtained by Bowen (5, 6) on angiosperms and *Equisetum*. The Benda method is most successful in the demonstration of the plastidome, although in the case of the pine, the pseudochondriosomes are also frequently well stained, especially if the differentiation of the crystal violet be not carried too far. The Kull method again stained the pseudochondriome most satisfactorily, sometimes alone, sometimes accompanied by a more or less extensive staining of the proplastids. Occasionally, however, especially in the promeristem, the Kull method yielded specific results of great clarity on the plastidome alone. None of the methods used gave very satisfactory results on the plastidome in the root-cap once the development of leucoplasts was well begun.

OBSERVATIONS.

The Oil-droplets.

Unless special technical attention is given to the oil-droplets these bodies usually constitute the most striking feature of the cytoplasm of the pine root-tip and seedling. Presumably they are to be assigned a meaning much the same as that given the lipoidal droplets so commonly, though not abundantly, present in the root-tips of angiosperms. We are apparently dealing here with lipoidal materials which are deposited in the cytoplasm as a result of the ordinary metabolic processes of the cell. In some cases these processes seem to yield a much larger quantity of lipoidal materials than is the case in others. The reasons for this variability are unknown, but the practical result is clearly evident in the pine. Since these oil-droplets are apt to occur in all kinds of preparations, and for our purposes have very little interest or importance, it is perhaps as well to consider them briefly at the very beginning in order to avoid further detailed reference to them.

The most marked accumulation of oil-droplets occurs in the promeristem of the root-tip. Here, after proper osmication by the Kolatchev method, the cells often show little or nothing else, being literally packed with oil-droplets (Pl. XXX, Fig. 20). They fill all of the interstices between the other cytoplasmic formed bodies, and are so closely crowded as often to be with difficulty distinguishable as separate droplets. These droplets are uniformly spherical in shape, but the size of individual droplets may vary through a considerable range. In this respect they differ from the similarly spherical pseudochondriosomes which never present any marked variability in size within a single cell. After osmication the droplets always have a characteristic brownish tone, and rarely or never acquire the jet-black colour which is the most striking feature of the action of osmic acid on many other cellular structures. The oil-droplets can thus be readily identified, and need not be confused with proplastids or pseudo-

chondriosomes that may be simultaneously demonstrated. Thus in Pl. XXX, Fig. 9, the plastidome is blackened together with the droplets (represented in the figures by a lighter tone); and in Pl. XXX, Fig. 35, the pseudochondriosomes are similarly blackened. In Pl. XXX, Fig. 23; Pl. XXXI, Figs. 43 and 44, the oil-droplets are only faintly visible in the presence of a blackening of other cytoplasmic constituents. In Pl. XXXI, Fig. 61, from the root-cap, and Pl. XXXI, Figs. 64 and 65, from the hypocotyl—both of a very young seedling—the pseudochondriome and plastidome respectively are blackened, while the oil-droplets are only browned as in the older root-tips. There is thus never any cause to confuse the droplets with other cytoplasmic components.

It may be noted that the droplets can be largely eliminated from the picture by bleaching. If this is not done the droplets often appear in Kull preparations as brown to yellowish bodies; and in the very young seedling they are sometimes stained intensely blue-purple by the crystal violet of Benda's method. Such preparations are usually of little use for cytoplasmic study.

As already observed, the oil-droplets are most abundant in the promeristem. Since some of the cells in this zone are always markedly elongate with a nucleus nearly as wide as the cell itself, the oil-droplets are frequently massed in two groups at the opposite ends of the cell. The resulting picture is a very characteristic one. During mitosis the droplets do not encroach upon the spindle-area, a feature often much emphasized in the late prophase where the chromosomal group and the polar caps are demarcated with remarkable sharpness by the boundaries of distribution of the droplets (Pl. XXX, Fig. 35).

As one passes from the promeristem, either down into the root-cap or up into the plerome and periblem, the droplets become rapidly thinned out, though still always very abundant. As the large vacuolar spaces of the differentiating cells are developed, the oil-droplets are carried outward with the peripheral lining of cytoplasm, and are never found within the vacuole-spaces. This feature is shown in Pl. XXXI, Figs. 38, 64, and 65. The enlargement of the cell and the consequent scattering of the cytoplasmic elements separates the oil-droplets, and in all kinds of older cells they are easily distinguishable as separate bodies.

The Vacuome.

Of the more permanent constituents of the cytoplasm, the vacuole apparatus may be dismissed with brief reference. This feature of the cell has been very extensively studied in gymnosperms by P. Dangeard (13), and we have given the vacuome very scant attention. It was found that the vacuoles of the more differentiated cells, especially in the root-cap, were

occasionally slightly blackened by osmic acid, or stained by thionin with the Kull method. Such results are, however, of little value so far as an understanding of the development of the vacuome from its primordia in the promeristem are concerned. We found, as a matter of fact, that the Kolatchev method rarely blackened the younger phases of the vacuome, and no results of any kind were ever obtained in the promeristem, plerome, or early root-cap. Possibly the Weigl method would have yielded more satisfactory results, as other work in this laboratory has shown to be the case with certain angiosperm root-tips. The only results obtained were in the outer layers of the periblem, where occasional success was met. Pl. XXXI, Figs. 58 and 59, are drawn from such impregnated areas. The vacuome consists of a collection of separate, more or less rounded or ovoidal vacuoles which are usually rendered in brownish-black or grey, rather than the jet-black sometimes obtained in angiosperm root-tips. Usually the impregnation is restricted to the vacuoles (Pl. XXXI, Fig. 59), but the pseudochondriosomes are also sometimes intensely blackened (Pl. XXXI, Fig. 58). Stages in the elongation and early fusion of these vacuoles to form the large vacuolar spaces of older periblem-cells were also observed.

These same periblem-cells frequently exhibit another type of response to osmic acid, the whole cell being a brown-yellow tone. The vacuolar outlines are then clearly visible, the vacuoles themselves being clear and transparent, and containing many yellowish bodies, presumably resulting from coagulation by the fixative. In Benda slides these bodies may be coloured a clear yellow, in contrast to the reddish nuclei and purple archiplasts. Pl. XXXI, Fig. 60 shows an early stage in the appearance of these intra-vacuolar bodies. In older cells, they are much more numerous. In the figure, the yellow of these bodies is rendered in a greyish tone. The archiplasts are also distinguishable, but as a rule their preservation in cells fixed in this way is far from perfect.

In older cells of all kinds the vacuoles appear only as the large, clear spaces, characteristic of all kinds of technical methods (Pl. XXXI, Figs. 38, 56, 57, 64, and 65).

The Root-tip¹—Plastidome and Pseudochondriome.

By means of the Benda and Kull methods of staining, there is brought to light in all kinds of cells² in the root-tip an assemblage of bodies in every way comparable to those described by Bowen (5, 6) in the root-tips of *Equisetum* and various angiosperms. In the pine, again, it is possible to effect a clear division of these bodies into two classes distinct morpho-

¹ This section deals only with young root-tips several millimetres in length. Root-tips of young seedlings which have not yet ruptured the seed-coat are considered in the section on seedlings, p. 579.

² In the dermatogen their demonstration is usually defective.

logically, functionally, and to some extent in their stainability. Of these two classes, one is clearly associated with the production of leucoplasts, and is therefore equivalent to the plastidome of other plants; the other is just as clearly not connected with plastid formation, and is obviously equivalent to what Bowen (4) has elsewhere tentatively termed the pseudochondriome.

The pseudochondriome consists, in all cells, of small bodies which are typically spherical, and during the interphase are distributed more or less evenly throughout the cytoplasm. It is sometimes possible by the Kull method to effect a complete separation of these granules from the plastidome, just as has been done in the angiosperms (5).

In Pl. XXX, Figs. 1 and 2, are shown cells from the general promeristem zone, prepared by the method of Champy-Kull. Only the pseudochondriosomes are demonstrated. For the most part they are clearly spherical, but occasionally more elongate forms occur. These have been frequently observed by other workers, and probably represent division-stages. Passing from the promeristem into the root-cap, the pseudochondriosomes retain at first the same appearance, but in the intermediate region, particularly of the lateral root-cap, the spherical forms are characteristically accompanied by many of more elongate type. Sometimes these are almost thread-like in shape (Pl. XXX, Figs. 16 and 17). That these elongate bodies have nothing to do with the plastidome is clear, from the fact that in the cells where they occur, starch-bearing leucoplasts have already been developed from the archiplasts of earlier cell-generations. In older cells (Pl. XXX, Fig. 19) the spherical form is again the more characteristic one. Possibly the thread-like shape of some of the pseudochondriosomes is associated with a period of very rapid multiplication. A similar tendency for the pseudochondriome to develop elongate forms has been noted especially by Guilliermond in the differentiating cells of angiosperms.

As a rule the Benda stain does not yield results on the pseudochondriome of the root-cap, but occasionally it succeeds. In such cases (Pl. XXX, Fig. 18) the pseudochondriosomes are selectively stained, and, as Bowen (5) has elsewhere observed, they appear to be smaller in size and less distinctly outlined than after the Champy-Kull technique.

The Kolatchev method yields frequent impregnations of the pseudochondriosomes in the older part of the root-cap, Pl. XXX, Fig. 19 being an extraordinarily clear example. In the younger cells, particularly those of the central portion of the root-cap, the pseudochondriosomes are often well blackened, but in that case usually associated with an accompanying demonstration of the oil-droplets (see Pl. XXXI, Fig. 61, from a very young root-tip). A similar result is also of frequent occurrence in the promeristem.

Returning now to the promeristem, and passing along the root-tip in the opposite direction, after the Kull stain the pseudochondriosomes appear

in similar form, and selectively stained in the various parts of the plerome and periblem (Pl. XXX, Figs. 29, 31, and 33; Pl. XXXI, Figs. 40, 47, 52, and 57). The predominant shape is the spherical one characteristic of the promeristem, and in the part of the root-tip which we studied, elongate forms like those in the root-cap were rare. Stages in the elongation and apparent 'binary fission' of a single pseudochondriosome are everywhere observable. After Kolatchev, the pseudochondriosomes are frequently blackened as in the root-cap, usually with, but clearly separable from, the oil-droplets (Pl. XXX, Fig. 35); but rarely together with the vacuome (Pl. XXXI, Fig. 58). Cases in which the plastidome is simultaneously blackened (Pl. XXXI, Fig. 53), are usually very complicated and difficult to decipher.

After the Mottier-Benda technique, the pseudochondriosomes in the promeristem and earlier plerome and periblem are never well demonstrated by themselves. On the other hand, frequent preparations are obtained, in which the pseudochondriome is well stained in conjunction with the archiplasts (Pl. XXX, Figs. 28, 30, 32, and 34; Pl. XXXI, Fig. 39). In such cases, as will be noted more fully beyond, the archiplasts are usually identifiable without trouble by their markedly different shape. It may also be observed that after Champy-Kull similar results are occasionally obtained (Pl. XXX, Figs. 24 and 25). These combination results are all of the greatest usefulness in effecting a positive identification and separation of these two classes of 'chondriosome-like' elements.

Throughout the region of the root-tip studied, the spherical shape of the individual pseudochondriosome remains a typical feature, and at no stage is there any evidence of functional activity which is reflected in important morphological changes. Neither is there the slightest evidence that these bodies are in any way associated with the plastidome. Indeed, in the older cells of plerome and periblem, the pseudochondriosomes remain unchanged alongside the leucoplasts which have arisen from the proplastids of the younger cells. The two kinds of bodies are always and everywhere distinguishable by their shape (and functional activity), the plastids being larger and much less numerous than the pseudochondriosomal granules (Pl. XXX, Fig. 36).

With respect to the structure of the individual pseudochondriosome, it is to be observed that we have frequently found the same vesicular structure noted particularly by Bowen (5) in the angiosperms.¹ We do not know positively whether this represents a normal structural feature, or is merely an artifact. It is similarly uncertain as to whether the granules in Benda and Kull slides present the exact dimensions of the pseudochondriosomes in the living cell, or may be constantly subject to a certain amount of swelling during fixation. At all events the relative constancy in size of

¹ This feature is rare in osmic preparations, the granules being typically solid black, with no suggestion of internal differentiation.

the granules by practically all the methods which we have used, argues something in favour of the view that our preparations are a close approximation of the condition in life. In this connexion it may also be noted that obvious swelling of the pseudochondriosomes sometimes occurs in Champy-Kull preparations, and rarely after Kolatchev. Two cases of the latter type (Kolatchev method) were observed in root-cap cells, one in the central cells, the other in the lateral region. In these the steps in the swelling of the granules to form larger vesicles with a heavily blackened rim could sometimes be easily followed. The figures thus produced have not the most remote resemblance to the osmiophilic platelets, which bodies Guilliermond (17) has recently asserted to be artifacts produced merely by the swelling of the pseudochondriosomes (his 'inactive chondriome'). Not only are the bodies produced by swelling of the pseudochondriosomes vesicular, and not plate-like, but they also present a considerable range of sizes within a single cell, which the osmiophilic platelets do not.

During mitosis, the pseudochondriosomes have been followed, as was done by Bowen (5) in angiosperms. And again, in the pine, there is no indication of any special orientation of the granules with respect to the spindle. The pseudochondriosomes are at all stages scattered more or less evenly through the cytoplasm, and the daughter-cells receive approximately equal amounts by virtue of this distribution. Various stages in the mitotic process are shown in Pl. XXX, Figs. 28, 30, 32, and 34; Pl. XXXI, Figs. 46 and 52. As in the case of angiosperms, there seems to be no special relation between the time of multiplication of the individual pseudochondriosomes and the division of the cell as a whole (mitosis).

Turning now to the second class of bodies which are demonstrable by the Benda and Kull staining methods, the plastidome may be similarly followed from the promeristem-area of the root-tip. The archiplasts or proplastids which constitute the plastidome of the promeristem-cells are always devoid of starch, exactly as in the angiosperms. The shape of the individual proplastids is very variable, and in the promeristem of different root-tips, or even in the same one, there may be considerable morphological variation. Similar conditions have been reported by Bowen (5) in *Vicia* and other angiosperms. In any case, however, the plastidome is demonstrable by the Benda method (Pl. XXX, Fig. 4), sometimes alone, sometimes accompanied by the pseudochondriome. It has also been found possible to stain the plastidome alone by the Kull method (Pl. XXX, Fig. 3), although this is more generally useful for demonstrating the pseudochondriosomes.

In the lowermost tip of the promeristem, the archiplasts occur in the form of a number of scattered bodies of variable shape. There is a tendency, however, toward a rather compact form, spindle-like, lenticular, or short rods (Pl. XXX, Fig. 4). Markedly elongate forms such as sometimes occur in the angiosperms are not the rule. In other parts of the promeristem in

the pine, however, the archiplasts are sometimes more thread-like. It is, further, not uncommon to find promeristems in which all of the archiplasts are of a very compact, often rounded type (Pl. XXX, Figs. 3 and 21), and similar forms are often extensively distributed through the young plerome (Pl. XXX, Fig. 24) and periblem (Pl. XXXI, Fig. 48). The meaning of this type of archiplast is not known, and it is possible that these rounded forms are at least in part the result of swelling during fixation.

In any event, as one passes into the plerome or periblem, sooner or later, and usually sooner, the archiplasts tend to become more elongate. At first they may assume various angular or fusiform shapes (Pl. XXX, Figs. 22 and 26; Pl. XXXI, Fig. 42), but these are soon stretched out, and frequently from the promeristem onward, the proplastids are quite thread- or rod-like in shape (Pl. XXX, Fig. 28; Pl. XXXI, Fig. 39). In neighbouring cells one often finds connecting stages in this elongation process, as shown, for example, in Pl. XXXI, Figs. 24 and 25, or Pl. XXXI, Figs. 48 and 49.

It will be clear from this somewhat confused picture that no definite statements can be made about the morphology of the proplastids in the meristem of the pine root-tip. Nevertheless, in spite of shapes running the entire gamut from spheres (Pl. XXX, Fig. 21) to threads (Pl. XXX, Fig. 28 and Pl. XXXI, Fig. 39), there is never any question as to the identification of these bodies and their separation from the much smaller pseudochondriosomes. In older cells, the thread-like form is frequently very marked (Pl. XXXI, Fig. 41). In some cases the archiplasts may become more compact (Pl. XXXI, Fig. 38), and are transformed into leucoplasts (Pl. XXX, Fig. 36), exactly as in the angiosperms. The stages in leucoplast-development in the older plerome and periblem were not studied by us in detail, but Nassonov (20) has given some account of it in *Pinus sylvestris*.

In Kolatchev preparations the plastidome, particularly in the promeristem and early plerome, is often intensely blackened. The results in general duplicate those obtained by staining with Benda or Kull, and are exactly comparable to those which have been obtained in the angiosperm root-tip by Bowen (5). Sometimes the plastidome alone is intensely blackened (Pl. XXX, Fig. 21), even the oil-droplets being completely invisible. At other times the oil-droplets may be partially demonstrated (Pl. XXX, Fig. 23; Pl. XXX, Fig. 43), and very frequently the osmiophilic platelets (Pl. XXX, Figs. 22, 23, and 27; Pl. XXXI, Fig. 43). A simultaneous blackening of plastidome and pseudochondriome is also occasionally obtained (Pl. XXXI, Fig. 53), but such results are usually very confusing and less satisfactory for study. On the whole, the results of osmic-acid impregnation of the gymnosperm root-tip are decidedly like those which Bowen (5) has described in the root-tips of angiosperms. In fact the figures drawn from examples of these two great plant-groups could be readily interchanged.

Before leaving the regions of the root-tip here under discussion, we should like to call attention to a special class of results sometimes, in fact frequently, obtained by either the Benda or Kull methods. We refer to cases in which both plastidome and pseudochondriome are stained together in the same cell. Bowen (5) found in angiosperms that the plastidome alone was usually stained by the Benda method; but in *Pinus* the results are more variable. While in many cases the plastidome alone is demonstrated, in other examples the pseudochondriosomes are very well stained by the crystal violet. Examples of this are shown in Pl. XXX, Figs. 28, 30, 32, and 34; Pl. XXXI, Figs. 39 and 46. Similar results are less frequent after the Kull stain (Pl. XXX, Figs. 24 and 25), but are of exactly the same nature when they do occur. Such preparations are of importance because they establish beyond question the simultaneous occurrence of both kinds of cytoplasmic bodies. Furthermore, and of much more importance, they offer a practically conclusive demonstration that these bodies are separable into two classes which can be recognized by their marked differences in morphology, and which present no clear indication of transition stages from one into the other. It is true that when both kinds of bodies are demonstrated simultaneously, the picture is very confusing, and in specific instances it is difficult at times to distinguish a fragment of an archiplast from a pseudochondriosome. Nevertheless, taking all the evidence into account, it is clear that the situation in the gymnosperm is exactly similar to that in the angiosperm and pteridophyte (compare Bowen 5, 6). We are dealing with two categories of bodies which differ in respect to their stainability and their reaction toward osmic acid, and in respect to their morphology, structure,¹ and function. So far as the situation in the pine is concerned, there is no critical evidence that the plastidome and pseudochondriome have any common chondriosomal basis as maintained by Guilliermond.

The conditions in the root-cap may be dismissed with brief mention, since they are in every way exactly comparable to those described by Bowen (5) in the angiosperm root-tip. In the earliest cells of the central root-cap, the proplastids are like those of the directly adjoining promeristem-tip (Pl. XXX, Fig. 5). In the youngest cells of the central columns, the proplastids remain usually in the form of short rods, or with slightly more compact shapes (Pl. XXX, Figs. 6, 7, and 9). The osmic methods work particularly well—and with their usual variations in type of result, while the Benda method is difficult to apply successfully. This tendency for the Benda stain to work indifferently on root-cap cells has also been noted in the case of angiosperms, but the causes are quite unknown. The archiplasts quickly become more rounded and proceed at once with their transformation into leucoplasts and the accompanying deposition of starch grains in

¹ As in the angiosperms, the archiplasts never present the vesicular appearance so characteristic of the pseudochondriosomes. They are always stained homogeneously.

their substance (Pl. XXX, Figs. 8, 10, 11, and 12). In the lateral root-cap cells the history is in essence the same, all the archiplasts being transformed into starch-bearing plastids (Pl. XXX, Figs. 13 and 14). In Fig. 15, older leucoplasts are shown. These and more advanced stages proved very refractory to all the methods which we tried. After the Kull stain particularly, we often received the curious refraction-images from the individual starch grains (Pl. XXXI, Fig. 62) which Bowen (5) observed in angiosperms. These permit one to locate leucoplasts in cases where plastid material itself is absolutely invisible. It may also be noted in passing that at times it was possible to stain both plastidome and pseudochondriome simultaneously (Pl. XXX, Figs. 12, 14, and 15). In such instances the identification of the two categories of bodies is beyond question.

With respect to the mode of multiplication of the archiplasts, it appears probable that they increase by a simple process of 'binary fission' as has often been described. Possible stages of such division are recognizable in many of our figures (e.g. Pl. XXX, Fig. 4; Pl. XXXI, Figs. 42, 48, 49, &c.). To this particular question we have, however, given no special attention.

The matter of how the plastidome behaves during mitosis has been of much more interest to us. Bowen (5, 7) has recently described and discussed a considerable range of cases in which the plastidome, as first clearly recognized by Nasonov (20), is oriented in a very special way with respect to the division-figure. In the root-tips of *Pinus* we have found the same essential phenomena, although in many cases it is not as clear as it is in favourable angiosperm examples. During the prophase the proplastids become aggregated into two groups which are arranged in a more or less clearly marked radial fashion at the nuclear poles (Pl. XXX, Figs. 26 and 28; Pl. XXXI, Fig. 42). It was of special interest to find that even when the archiplasts were more compact, their orientation was still clearly recognizable in some cases at least (Pl. XXXI, Fig. 42). There was also some indication that the archiplasts tend to be drawn out into more elongate shapes during their prophase-orientation, exactly as Bowen (7) has observed in the angiosperms. The subsequent division-stages (Pl. XXX, Figs. 30, 32, and 34; Pl. XXXI, Figs. 43 and 46) are passed through as in the angiosperms, the daughter-cells each receiving about half of the original plastidome (Pl. XXX, Fig. 37). It is a curious fact that during the later division-phases the proplastids very frequently assume a ring-like shape. These rings may be relatively scarce in a given cell (Pl. XXX, Figs. 30 and 34), but sometimes dominate the whole picture (Pl. XXX, Fig. 37).¹ This extraordinary behaviour of the plastidome during the later stages of mitosis is exactly paralleled in some at least of the angiosperms (5). What such a similarity may mean, occurring as it does in plants so widely different, is entirely obscure, as indeed is the meaning of the phenomenon itself.

¹ Compare, for example, with Fig. 69 from *Vicia* (Bowen, 5).

The Osmiophilic Platelets.

There remains for consideration a category of cytoplasmic bodies to which Bowen (2, 3) has applied the tentative name of osmiophilic platelets. These he has described in some detail in Bryophyta, Pteridophyta, and Angiospermae. Thus far there are no reports of the bodies in Gymnospermae. By the use of the Kolatchev method we have succeeded in demonstrating the platelets in all kinds of cells in the root-tip of *Pinus*. In their reaction to osmic-acid impregnation and other staining methods, their morphology, their behaviour in mitosis, and their apparent lack of any function expressible in morphological changes, in all these features the platelets in *Pinus* are exactly the same as those in the other plant-groups which Bowen, and later Patten, Scott, and Gatenby (21), have studied. In one respect, however, the gymnosperm material proved much more refractory than is the case with many angiosperms. We refer to the difficulties introduced by the great number of oil-droplets which are always present. In many of the trials these effectually frustrated any satisfactory demonstration of the platelets, but sometimes good impregnations are obtained over a more or less extensive area. The best results, however, were obtained in roots from seeds germinated in sand, under which circumstances the oil-droplets were frequently eliminated from the resulting picture, allowing a quite satisfactory demonstration of the platelets alone.

The platelets appear in all areas of the root-tip in characteristic form—as sharply blackened rings which are circles in plane view, and ellipses or rods according as they are seen obliquely or in profile. As regards the number of platelets per cell, they correspond to the situation commonly met in the angiosperms. In general, a cell from the root-tip of *Pinus* and one from an angiosperm root-tip would be morphologically interchangeable.

Examples of the impregnation of the platelets alone are shown in Pl. XXXI, Fig. 45, from the border zone of root-cap and periblem, and in Pl. XXXI, Figs. 54, 55, and 56, from various zones of the periblem. In Pl. XXXI, Fig. 56, the formation of the vacuole-spaces is far advanced, and the platelets are retreating toward the cell periphery along with the cytoplasm, exactly as in the angiosperms. In the promeristem and early plerome successful impregnation of the platelets is almost always accompanied by a good impregnation of the plastidome—again precisely the result observed in the angiosperm root-tip by Bowen (4). Examples of this result are shown in Pl. XXX, Figs. 5, 22, and 23, and much less successfully in Pl. XXXI, Fig. 43. In Pl. XXX, Figs. 23 and Pl. XXXI, 43, the oil-droplets also appear faintly, and a similar result is shown in Pl. XXXI, Fig. 44, where the plastidome is invisible. Pl. XXX, Fig. 5, from the tip of the promeristem, is characteristic of one type of result in which the archiplasts are but lightly blackened, while the platelets are well impregnated. A similar

result in a plerome-cell is shown in Pl. XXX, Fig. 27. In Pl. XXXI, Fig. 51, from the periblem, the plastidome is heavily blackened, together with some darkening of oil-droplets, while in Pl. XXXI, Fig. 53, there is a blackening of at least a part of plastidome, platelets, and pseudochondriosomes. In the younger root-cap results of similar nature to those in other parts of the root-tip were obtained, especially in the central columns of cells (Pl. XXX, Figs. 6 and 8).

In rather rare instances a good impregnation of the platelets was achieved, together with the usual intense browning of the oil-droplets. A tangential section of a cell in which this had occurred is shown in Pl. XXXI, Fig. 50, from the young periblem. Such preparations, taken in conjunction with those in which the oil-droplets are not even visible and with those from angiosperms in which lipoidal droplets are relatively scarce, are ample proof of the absolutely separate nature of the platelets and lipoidal or oily droplets.

The behaviour of the platelets during mitosis (Pl. XXXI, Fig. 44) was not particularly studied, but so far as our observations go, they indicate no important differences from the phenomena observed in detail by Bowen (4) in angiosperms. It may be of interest to record the fact that particularly during the metaphase the spindle-fibres associated with chromosomes are sometimes selectively blackened, as first observed by Nassonov (20) in *Vicia*, and since corroborated by Bowen (8) and others. In *Pinus*, however, the results which we obtained were never as clear-cut as they sometimes are in angiosperms. In this connexion it may also be noted that the total blackening of the nuclei, or of intra-nucleolar bodies, so commonly met with in angiosperms (Bowen, 8) was not observed in the pine root-tips.

Young Seedlings.

Our results on the young seedlings removed from the seed just before or soon after the breaking of the outer seed covering are by no means so complete as those for the root-tips. This is due entirely to the fact that the material is technically much more difficult to handle. Nevertheless, in general it is perfectly clear that the conditions in the seedling are a replica of those in the root-tips already described.

The pseudochondriosomes occur in the root-cap area of very early seedlings (Pl. XXXI, Figs. 61 and 62) exactly as in the root-cap of older tips, and presenting the same technical responses. In Pl. XXXI, Fig. 62, the leucoplasts can also be distinguished, some of them showing the characteristic refraction effects produced by the starch grains already deposited in the plastid substance. In the promeristem and adjoining regions of the root-tip area the fixation was always too defective to permit the proper demonstration of the pseudochondriome. In the hypocotyl (Pl. XXXI,

Figs. 64 and 66) the pseudochondriosomes occur again in characteristic form. They are often impregnated by the osmic treatment (Pl. XXXI, Fig. 64), although frequently in somewhat distorted form. In such preparations the oil-droplets are always heavily browned (Pl. XXXI, Fig. 64). After Champy-Kull the plastids are usually also stained (Pl. XXXI, Fig. 66). The pseudochondriosomes were observed in practically all parts of the hypocotyl, including the epidermis. Similar results were not obtained in the growing-point itself or in the leaf-rudiments because of inadequate fixation. In the cotyledons the pseudochondriosomes occur exactly as in the hypocotyl. In all these cells the pseudochondriosomes are sometimes demonstrated together with the plastidome by the Benda stain. Such a result is shown in Pl. XXXI, Fig. 67, from the basal region of a cotyledon. As earlier noted in the case of the root-cap, the pseudochondriosomes in these cases often appear smaller and less clearly defined than with the Kull technique.

In the hypocotyl and cotyledons the plastidome occurs in the epidermis in the form of rather elongate bodies, which in the deeper layers have become transformed into obvious plastids (Pl. XXXI, Figs. 65 and 66). At the base of each cotyledon is a region where some of the steps in the transition are frequently demonstrated (Pl. XXXI, Fig. 67). In the hypocotyl there is one particular zone of elongate cells, which seem to form a continuation of the endodermis-pericycle of the root-tip, in which the development of the plastids is most extraordinary (Pl. XXXI, Fig. 68). The plastidome, in parts at least of hypocotyl and cotyledons, is rather easily and regularly stained either by Kull or Benda. In some cases it is also intensely blackened by osmic acid, in which case the oil-droplets are also heavily browned (Pl. XXXI, Fig. 65). In the growing-points, and more frequently and successfully in the early leaf-rudiments which surround it, the plastidome was impregnated by the Kolatchev method. Usually some defective blackening of other bodies also occurs, but the general morphology and disposition of the plastidome is still clear. The plastidome in these meristematic cells is in the form of many scattered archiplasts (Pl. XXXI, Fig. 63), which are roughly comparable in shape to some of the types found in the older root-tips.

The impregnation of the osmiophilic platelets proved almost invariably unsuccessful. Apparently the great abundance of oil-droplets introduced a fatal obstacle. Nevertheless, in the leaf-rudiments, some more or less defective results were obtained. A few platelets can be identified in Pl. XXXI, Fig. 63, and it is clear that at least in these cells the platelets are present in characteristic form. It may be observed that in many other parts of the seedling after the Kolatchev method the pseudochondriosomes are not infrequently demonstrated in distorted form, but these distortions would not be identified as platelets by one who was familiar with the appearance of the latter after proper fixation. This is a point of some

interest in view of Guilliermond's (17) recent assertion that the platelets are nothing but distorted pseudochondriosomes.

DISCUSSION.

A comparison of the results here recorded on the structural elements of the cytoplasm in gymnosperms with those previously recorded by the senior author on other plant groups can scarcely elicit any surprise, except perhaps in one particular. This is the amazing similarity in the morphology of the various categories of cytoplasmic bodies throughout so large and varied a range of plant-cells. Not only is there the constant and expected recurrence of certain classes of formed elements, but within rather narrow limits the morphology of these classes is substantially identical in pteridophyte, gymnosperm, and angiosperm. Indeed, the only discordant point is the matter of the shapes assumed by the proplastids in meristematic tissue. To one accustomed to the wide variation in the form of the chondriome and Golgi apparatus so characteristic of animal-cells, this constancy in shape of the cytoplasmic bodies of plant-cells seems most extraordinary. In the gymnosperm-cell, again, the osmiophilic platelets occur exactly as in *Polytrichum* (Bowen, 2), *Equisetum* (Bowen, 4), and the angiosperms (Bowen, 4, and Patten, Scott, and Gatenby, 21). Similarly the 'chondriosome-like' bodies are divisible into the customary two classes, of which the one—pseudochondriosomes—consists always of many scattered granules which are normally spherical in meristem cells; while the other—archiplasts or primordial plastids—consists of an assemblage of bodies of more varied form, but showing a tendency to be elongate, rarely spherical, and becoming always transformed into plastids. So also in the case of the vacuome, as demonstrated more adequately by the work of Dangeard (13), the same types occur as are found in the angiosperms. Briefly, then, our conclusion is that in the gymnosperm-cell the morphological organization of the cytoplasm is the same as that described by Bowen in the other higher plant groups. In a general way, and in practically all respects as regards details, the gymnosperm-cell is a replica of the angiosperm-cell. Only in the abundance of its oil-droplets does the pine-cell present any striking difference, and even this is probably one of degree rather than kind.

The cytoplasm of the gymnosperms has attracted the attention of relatively few modern students of the cell. Only by Dangeard (13) has the subject been investigated in any detail.¹ This author mentions briefly some older observations which are, however, of minor interest for the present-day worker.

Dangeard made an extensive examination of the embryo and young

¹ Dangeard published a series of brief preliminary notes (9, 10, 11, 12, 14, 15) touching on various points which were eventually described in detail in his final paper on the subject in 1923 (18).

seedling of several gymnosperms, also of the pollen and its development, from the standpoint particularly of *intra vitam* study of the vacuome. These studies were supplemented by examination of parallel material fixed by the customary Regaud method and stained with Fe-hematoxylin. His report on the vacuome as revealed by vital staining is very extended, but in respect to the other constituents of the cytoplasm his findings are given in a disappointingly abbreviated form, especially as regards the figures. It is clear, however, that our observations are in essential agreement with the conclusions of Dangeard so far as the 'chondriosome-like' bodies are concerned.¹ Dangeard found in a considerable variety of cell-types from several gymnosperms that these bodies divided themselves readily into two classes which he believed were entirely independent. One of these, his plastidome, corresponds obviously with our class of the same name. His figures from living cells usually show the archiplasts somewhat more regular in shape, less numerous and smaller than are the same bodies in our preparations. His most detailed figures, however, present the plastidome in a form not unlike that which we have sometimes found in the tip of the promeristem. In spite of these slight discrepancies it is obvious that our conclusions substantiate Dangeard as regards his general conception of the plastidome. In respect to the other class of these 'chondriosome-like' bodies, his sphérome as obviously corresponds to our pseudochondriome. As regards the probable normal shape of these bodies in the living cell and their complete independence of the plastidome, our conclusions again substantiate Dangeard in all important respects. In a similar way our conception of the oil-droplets coincides with that of Dangeard. These two sets of observations covering in detail somewhat different aspects of the gymnosperm-cell thus supplement and corroborate each other as to the morphology and separate identity of the plastidome and pseudochondriome (sphérome). Dangeard did not of course mention the osmiophilic platelets which we have here for the first time described in gymnosperms.

Other recent workers on the cytoplasm of gymnosperms seem to have made observations only on the 'chondriosome-like' bodies.² The most interesting study is that of Mottier (19), who found in the hypocotyl of *Pinus Banksiana* conditions similar to those we have found in the same kind of cells. By the Benda method he stained the plastidome and pseudochondriome simultaneously, with results akin to our Pl. XXXI, Fig. 67. The small granules he called chondriosomes, and considered them as a class of cytoplasmic bodies separate from the plastidome. His results on the

¹ Disregarding any views which Dangeard may have held in regard to the possibilities of *de novo* origin of the bodies in question. Our observations tend, indeed, to support the view that these bodies arise only by division of pre-existing elements of the same kind, but obviously we have attempted no critical examination of this point, and do not wish to express any opinion on the matter at present.

² In addition to the references here discussed, see also Moreau (18).

growing-point of the stem in young seedlings do not seem in harmony with our findings or those of Dangeard. He appears to have made an erroneous identification of the archiplasts, probably through the inadequacy of his technique.

In *Taxus*, Pensa (22) reports the occurrence of chlorophyll-bearing archiplasts and plastids in the growing-point and adjacent leaf-rudiments. His figures from the growing-point show archiplasts of varied shape, approaching more nearly our findings than the regularly lenticular bodies figured by Dangeard. Pensa traced the development of these bodies into typical plastids. He does not mention the pseudochondriosomes which his method (of reduced silver) was not adequate to demonstrate. Nassonov (20) in the root-tips of *Pinus sylvestris* has followed the transformation of thread-like archiplasts, apparently like those of our Pl. XXXI, Fig. 4I, into leucoplasts. He does not mention the pseudochondriosomes, which were apparently not demonstrated in his preparations. These observations fit obviously into the scheme suggested by our conclusions, although Nassonov identified the plastidome with chondriosomes. Pensa left the question of their homology undecided, as we have preferred to do for the present at least.

More recently Devisé (16) and Prosina (23) have reported on the microsporocytes of *Larix*. Unfortunately they did not make any distinction between the two classes of 'chondriosome-like' bodies, but identified them indiscriminately as 'chondriosomes'.¹ Their studies do not, therefore, throw much light on the problems in which we have been more particularly interested.

SUMMARY.

1. A study of the formed elements in the cytoplasm of several species of *Pinus* reveals the presence of the same structural components as have been found by the senior author in the other higher groups of plants.

2. These are (1) plastidome, (2) pseudochondriome, (3) vacuome, (4) osmiophilic platelets, (5) oil-droplets.

3. The morphology and behaviour of these various elements has been found to agree almost exactly with their characteristic features in other groups of plants.

4. The osmiophilic platelets are described in gymnosperms for the first time. The evidence again indicates strongly that these platelets are independent cytoplasmic bodies, and are not mere distortions of other previously known elements, as has been recently held by Guilliermond.

5. Our conclusions as regards the separate nature of the plastidome and pseudochondriome agree in general with the findings of P. Dangeard.

¹ Some of the figures, particularly in the paper by Devisé, indicate the presence of both spherical and elongate types, which perhaps correspond with the pseudochondriome and plastidome.

Further, our observations again emphasize the point that in meristem tissues no critical evidence can be found for identifying any particular cytoplasmic bodies as the homologue of the chondriosomes in animal tissues.

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EXPLANATION OF PLATES XXX AND XXXI.

Illustrating Professor R. H. Bowen's and Mrs. L. H. Buck's paper on Cytoplasmic Structure in Gymnosperms.

All of the figures have been outlined as far as possible with the camera lucida at an initial enlargement of approximately 1,675 diameters, and subsequently corrected and completed free-hand. In reproducing, the figures have been reduced uniformly to an enlargement of approximately 1,250 diameters. All the cells from root-tips are printed with that side toward the top which was originally directed toward the seed; similarly, in the case of seedlings all cells are printed with that side toward the top which was originally directed toward the growing tip (except Fig. 62, the top of which is at the left). The method employed in the preparation of the original object is appended to the explanation of each figure, details being given according to the formulae explained on page 706 of the first study on plant protoplasm by Bowen (4) and extended in the second study (Bowen, 5); see also the third footnote on page 566 of the present paper.

EXPLANATION OF FIGURES.

PLATE XXX.

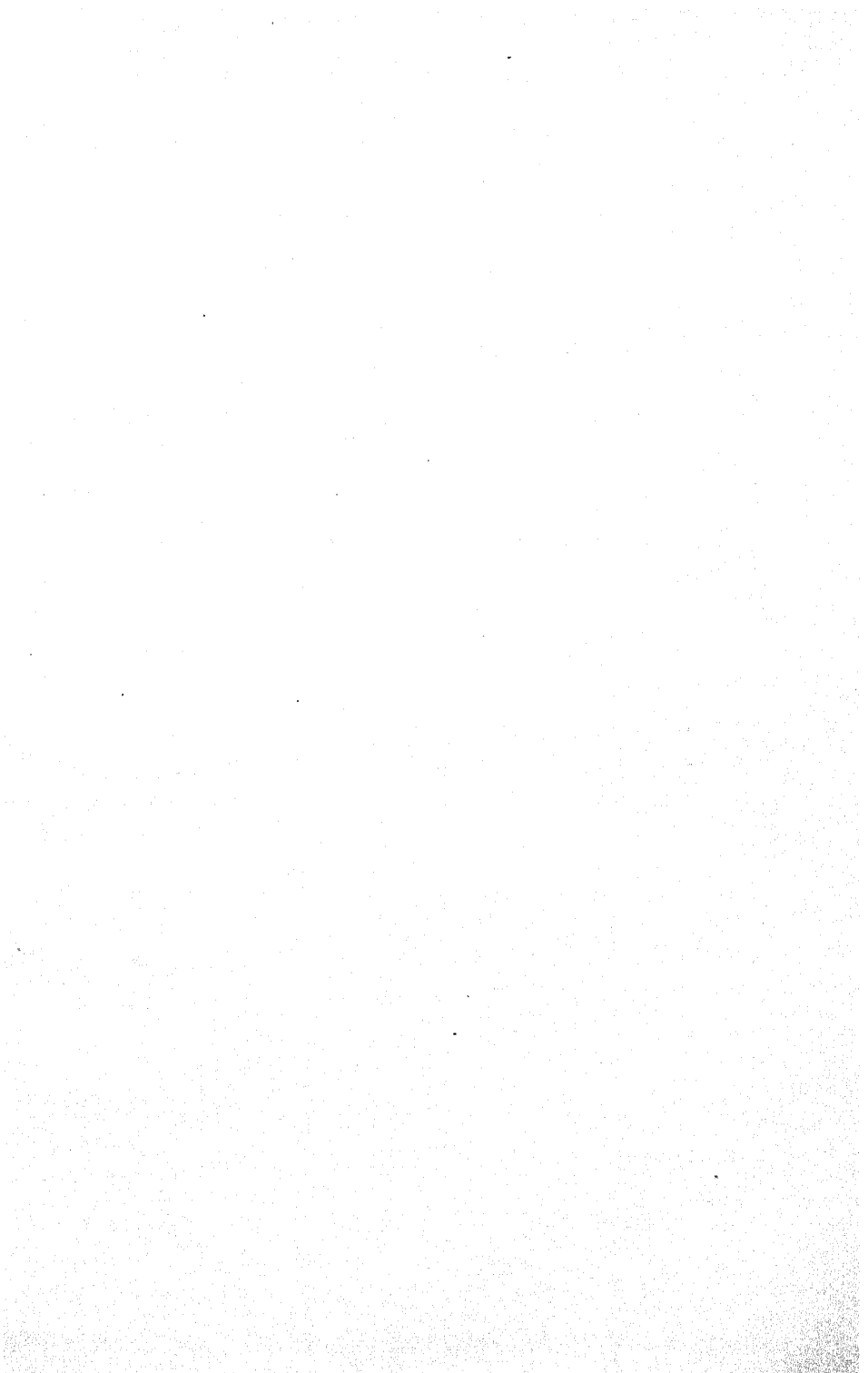
All the figures are from young, main root-tips of *Pinus*. Figs. 1, 2, 4, 9, 14, 15, 23, and 29 to 34 are from *P. Mughus*; Figs. 3, 5, 6, 7, 8, 10 to 13, 16 to 18, 21, 22, 24 to 28, 36, and 37 are from *P. austriaca*; Figs. 20 and 35 are from *P. sylvestris*; and Fig. 19 from *P. edulis*.

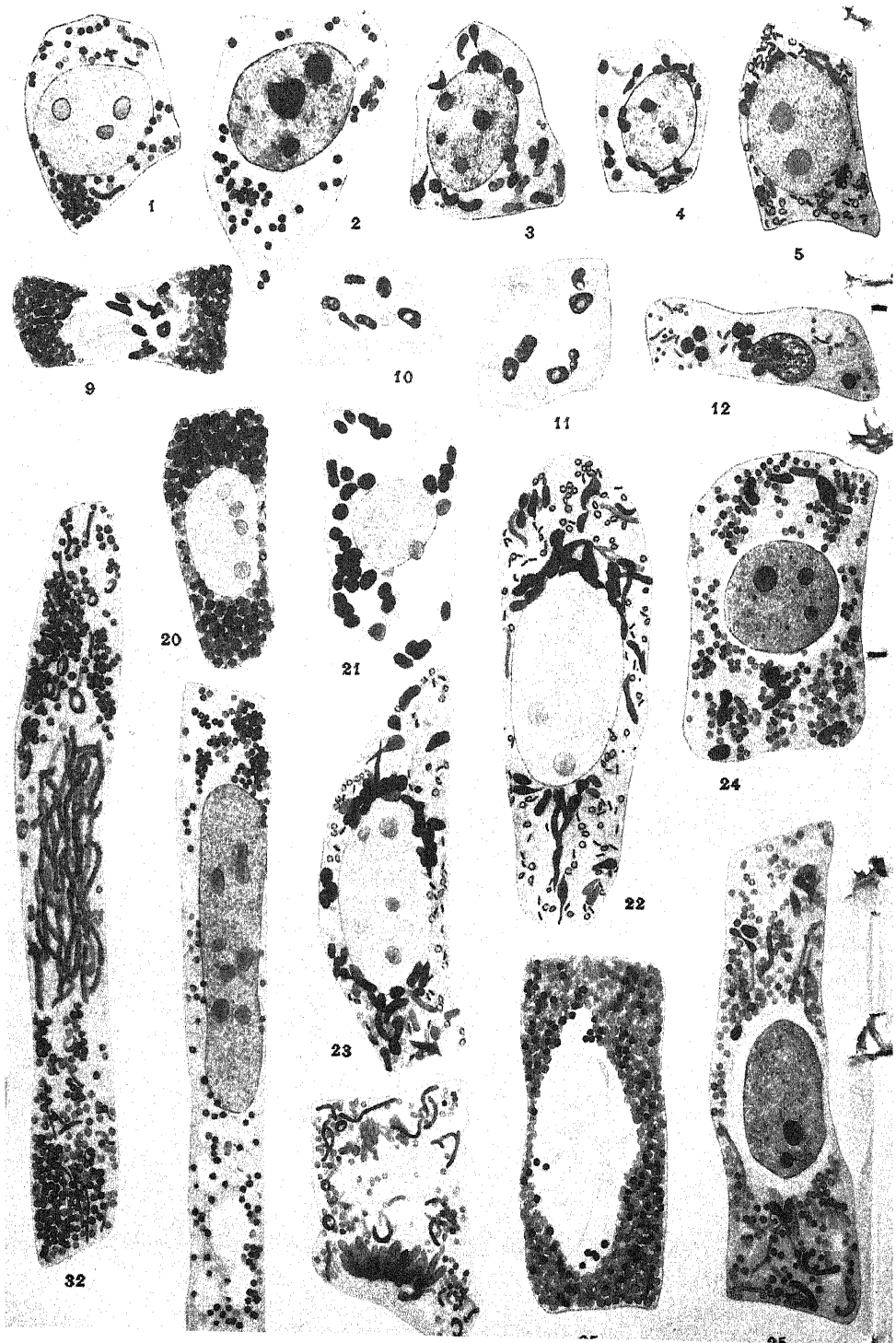
- Figs. 1 and 2. Promeristem. S-Champy-Kull.
 Fig. 3. Promeristem. M-Champy-Kull.
 Fig. 4. Lowermost tip of promeristem. S-Mottier-Benda.
 Fig. 5. Junction between promeristem and central part of root-cap. K-S ($2\% - 40^\circ : 35^\circ$) 5.
 Fig. 6. Earliest central portion of root-cap. K-S ($2\% - 40^\circ : 35^\circ$) 5.
 Fig. 7. Central part of root-cap. K-M ($2\% - 40^\circ : 35^\circ$) 5.
 Fig. 8. Central part of root-cap. K-S ($2\% - 40^\circ : 35^\circ$) 5.
 Fig. 9. Early central part of root-cap. K-M ($2\% - 40^\circ : 35^\circ$) 7.
 Figs. 10 and 11. Transformation of proplastids into leucoplasts in central portion of root-cap. M-Champy-Kull.
 Fig. 12. Same as preceding figure. S-Mottier-Benda.
 Figs. 13 and 14. Development of leucoplasts in lateral region of root-cap. M-Champy-Kull.
 Figs. 15 to 17. Lateral region of root-cap. M-Champy-Kull.
 Fig. 18. Root-cap. S-Mottier-Benda.
 Fig. 19. Outer portion of lateral root-cap; the large, mottled areas are vacuoles. K-M ($2\% - 35^\circ$) 7.
 Fig. 20. Lateral region of promeristem. K-M ($2\% - 40^\circ : 35^\circ$) 5.
 Fig. 21. Border between promeristem and plerome. K-M ($2\% - 40^\circ : 35^\circ$) 5.
 Fig. 22. Same as preceding figure. K-S ($2\% - 40^\circ : 35^\circ$) 5.
 Fig. 23. Promeristem-plerome border of early plerome. K-M ($2\% - 40^\circ : 35^\circ$) 7.
 Figs. 24 and 25. Two cells in the same column of plerome-cells, Fig. 24 being close to the promeristem, and Fig. 25 farther along the plerome. M-Champy-Kull.
 Fig. 26. Late prophase from the plerome. S-Mottier-Benda.
 Fig. 27. Plerome. K-S ($2\% - 40^\circ : 35^\circ$) 5.
 Fig. 28. Prophase from the early plerome. S-Mottier-Benda.
 Fig. 29. Plerome. S-Champy-Kull.
 Fig. 30. Early telophase stage from the young plerome. S-Mottier-Benda.
 Fig. 31. Plerome. S-Champy-Kull.
 Fig. 32. Division stage, probably an early anaphase, in the plerome. S-Mottier-Benda.
 Fig. 33. Older plerome cell. S-Champy-Kull.
 Fig. 34. Final anaphase from the outer plerome. S-Mottier-Benda.
 Fig. 35. Prophase stage from the outer plerome. K-M ($2\% - 40^\circ : 35^\circ$) 8.
 Fig. 36. Cytoplasmic fragment from an old plerome-cell. M-Champy-Kull.
 Fig. 37. Late telophase from plerome-periblem border. S-Mottier-Benda.

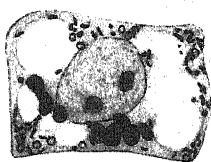
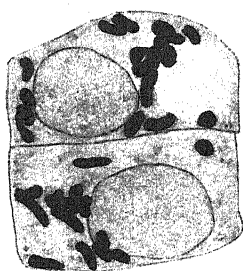
PLATE XXXI.

Figs. 38 to 60 inclusive are from young, main root-tips of *Pinus*; and Figs. 61 to 68 are from seedlings of *Pinus* at the beginning of germination. Figs. 40, 41, 43, 46, 47, 52, 54, 55, 57 to 60, and 65 to 68 are from *P. Mughus*; Figs. 38, 39, 42, 44, 45, 48 to 51, 53, 56, 61, and 62 are from *P. austriaca*; and Figs. 63 and 64 are from *P. sylvestris*.

- Fig. 38. Older plerome. K-M ($2\% - 40^\circ : 35^\circ$) 7.
 Fig. 39. Plerome-periblem border. S-Mottier-Benda.
 Fig. 40. Plerome-periblem border. S-Champy-Kull.
 Fig. 41. Older plerome-periblem border. S-Mottier-Benda.
 Fig. 42. Late prophase from plerome-periblem border. S-Mottier-Benda.
 Fig. 43. Telophase; plerome-periblem border. K-M ($2\% - 40^\circ : 35^\circ$) 7.
 Fig. 44. Middle telophase from plerome-periblem border. K-S ($2\% - 40^\circ : 35^\circ$) 7.
 Fig. 45. Border between lateral root-cap and periblem. K-S ($2\% - 40^\circ : 35^\circ$) 7.
 Fig. 46. Anaphase, from same zone as preceding figure. S-Mottier-Benda.
 Fig. 47. Young periblem. S-Champy-Kull.
 Figs. 48 and 49. Periblem or plerome-periblem border. S-Mottier-Benda.
 Fig. 50. Younger periblem. K-M ($2\% - 40^\circ : 35^\circ$) 6.
 Fig. 51. Periblem. K-M ($2\% - 40^\circ : 35^\circ$) 7.
 Fig. 52. Anaphase from inner periblem. M-Champy-Kull.
 Fig. 53. Inner periblem. K-M ($2\% - 40^\circ : 35^\circ$) 7.
 Fig. 54. Periblem. K-M ($2\% - 40^\circ : 35^\circ$) 7.
 Fig. 55. Inner periblem. K-S ($2\% - 40^\circ : 35^\circ$) 7.
 Fig. 56. Old periblem. K-S ($2\% - 40^\circ : 35^\circ$) 5.
 Fig. 57. Old periblem. S-Champy-Kull.
 Fig. 58. Outermost periblem. K-S ($2\% - 40^\circ : 35^\circ$) 3.
 Fig. 59. Outer periblem. K-S ($2\% - 40^\circ : 35^\circ$) 5.
 Fig. 60. Outer periblem. S-Mottier-Benda.
 Fig. 61. Central portion of a very young root-cap. K-M ($2\% - 40^\circ : 35^\circ$) 8.
 Fig. 62. Portion of cell from lateral region of a very young root-cap. M-Champy-Kull.
 Fig. 63. Cell from apical border of a very young leaf-rudiment. K-M ($2\% - 40^\circ : 35^\circ$) 7.
 Fig. 64. Cell from hypocotyl. K-M ($2\% - 40^\circ : 35^\circ$) 7.
 Fig. 65. Cell from hypocotyl, from layer just below the epidermis. K-M ($2\% - 40^\circ : 35^\circ$) 8.
 Fig. 66. Portion of an older cell situated like the preceding figure. M-Champy-Kull.
 Fig. 67. Cell from the base of a cotyledon, from layer just below the epidermis. M-Mottier-Benda.
 Fig. 68. Cell from hypocotyl, located in continuation of pericycle-endodermis of the root-tip. M-Mottier-Benda.







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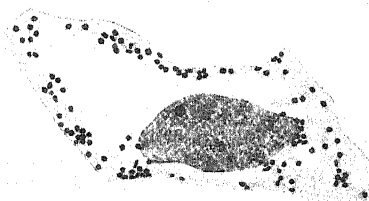
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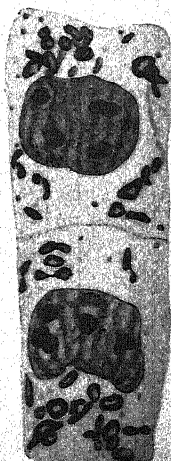
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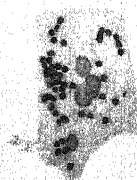
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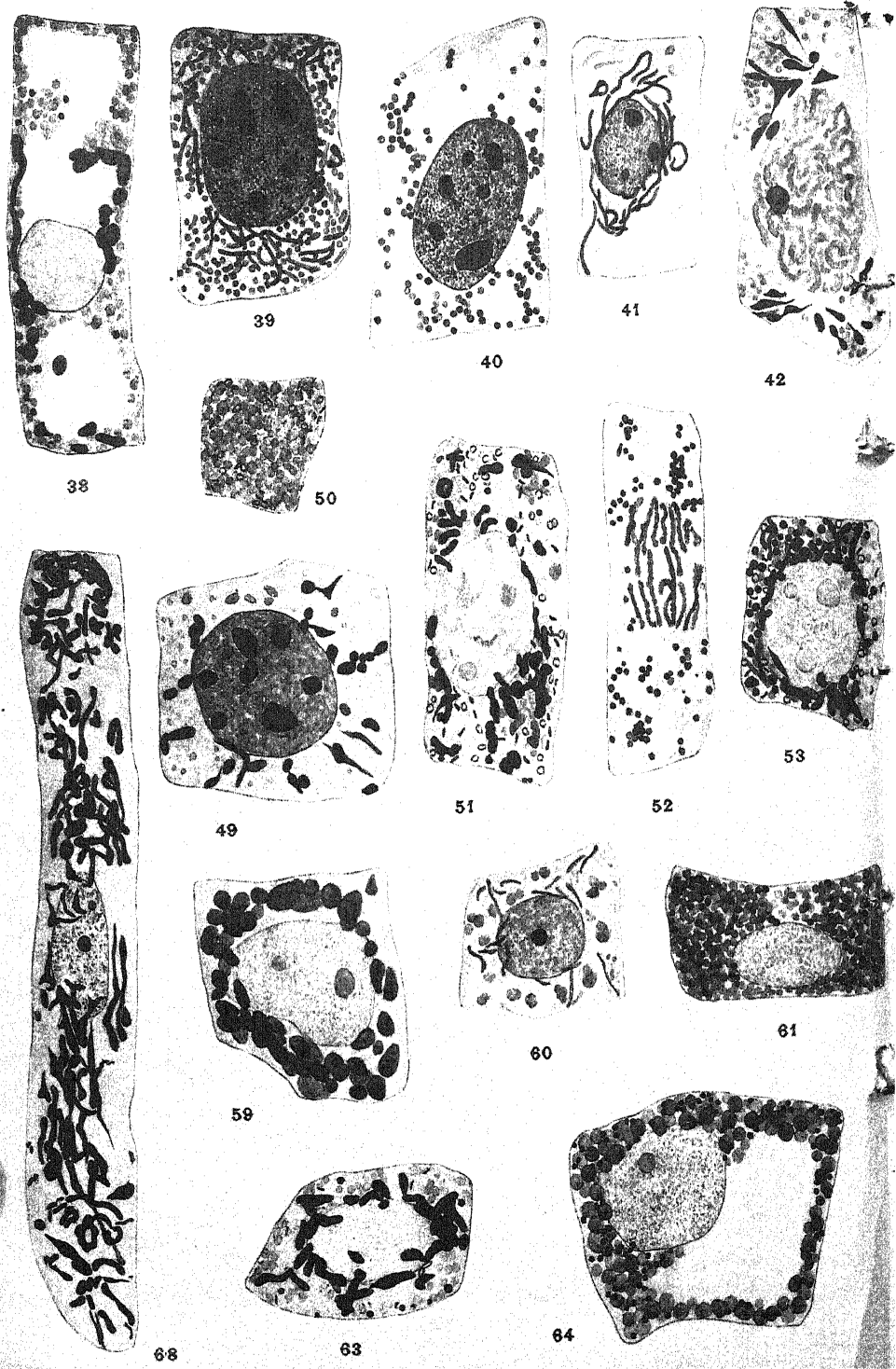
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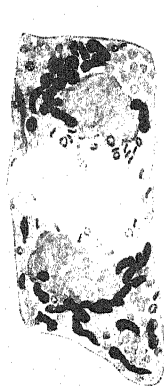


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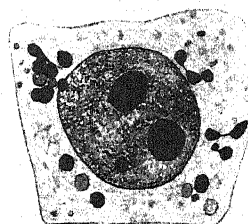
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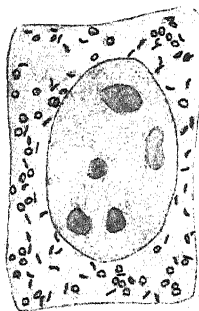
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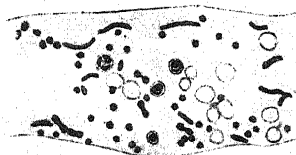
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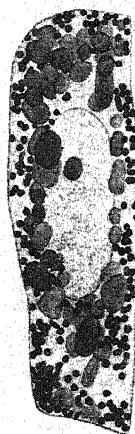
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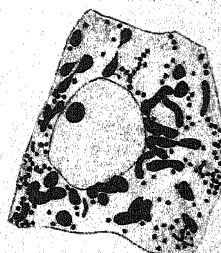
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Reduction Division in a Species of *Cladophora*.

BY

E. MARION HIGGINS, B.Sc., PH.D.

(University Department of Botany, Durham.)

With Plate XXXII.

IN the summer of 1928, on examining a collection of slides which had been made for class purposes two years previously, a preparation¹ of a species of *Cladophora* was found which showed all stages of nuclear division. The material consists of an unsectioned portion of a plant which had been stained in Heidenhain's iron haematoxylin. Although there is not sufficient material to make possible an exact identification of the species, its morphological features agree in most essentials with a species which was being worked on by a former student at Liverpool at the time the preparation was made, i.e. *C. flavescentis*, but in which the cytology was not worked out.

The investigation was begun in the Botanical Department of the University of Liverpool, and a preliminary account of the cytology was given to Section K of the British Association at Glasgow in September, 1928. The work has been completed in the University Department of Botany at Durham.

The plant bears sporangia in all stages of development, and the nuclear history of both the sporangia and of the vegetative parts is clearly demonstrated.

The number of nuclei in each section of the filament is higher than that usually recorded for species of *Cladophora*, and varies considerably, but is usually of the order of 30 or 60, and may reach 120 or more in the sporangia.

In the resting condition the nuclei stain very lightly with the exception of the nucleoli, of which there is usually one in each nucleus, although two are occasionally present. In the rare cases in which two nucleoli occur the nucleus appears larger and slightly elongated.

When the nucleus is about to divide mitotically it enlarges, and its

¹ The preparation had been made for the purpose of demonstrating the coenocytic nature of the thallus to junior students, and there is no record of the fixative used. It was certainly, however, either 50-70 per cent. alcohol or else a chrom-acetic mixture.

outlines become very much more distinct. The chromatin begins to appear as distinct dots and discontinuous threads scattered throughout the whole nuclear cavity (Pl. XXXII, Fig. 1). At a very early stage the chromosomes differentiate into very much elongated, deeply staining bars. Even at such an early stage as that shown in Pl. XXXII, Fig. 2, the limits of the individual chromosomes are distinguishable, and in no case has a continuous spireme, such as that described by Dr. Carter (1) for *C. glomerata*, var. *simplicior* (Kutz), been seen. At this stage, too, the nuclear membrane has disappeared, and the subsequent stages of the division of the nucleus take place within a rather poorly defined colourless area within the network of the chloroplast.

The chromosomes next thicken and become more distinct from one another and scattered throughout the nuclear cavity. At this stage they are most easily counted, and have been found to be 24 in number (Pl. XXXII, Fig. 3). At a slightly later stage each of these thickened chromosomes is seen to have undergone a longitudinal division. In Pl. XXXII, Fig. 4, most of the chromosomes show this longitudinal fission, although a few remain as the homogeneous thickened bars. Pl. XXXII, Fig. 5, shows the resulting pairs of half chromosomes in a nucleus in which all the chromosomes have divided. In the early stages of fission, bridges of chromatic material are frequently seen between corresponding chromomeres in the two halves of a divided chromosome. When the fission is complete, the two daughter chromosomes are commonly seen to be twisted round one another one or more times. Divided chromosomes from different nuclei are seen on a larger scale in Pl. XXXII, Fig. 6, in which the bridges of chromatin and the twisted chromosomes are shown.

The stage in which fission occurs appears to represent the metaphase of the division, since the chromosomes all lie approximately in one plane, but no spindle has been seen, due possibly to the fixation. The halves of the divided chromosomes move apart and become narrower and somewhat more elongated. Pl. XXXII, Fig. 7, shows them passing to the poles in anaphase, each chromosome orientated at right angles to the plane of division. Two groups are formed, they become rounded off, and the two daughter nuclei are organized, a nucleolus making its appearance in each (Pl. XXXII, Fig. 8).

The foregoing division stages agree in detail, although not in chromosome number, with those figured by T'Serclaes for *C. glomerata* (4).

REDUCTION DIVISION.

Sporangium formation takes place in basipetal succession from the terminal portions of the filaments, and is preceded by a reduction division.

Usually all the nuclei in each section of the filament are found to be

in the same stage of division at the same time, although occasionally all stages may be found together side by side. Until the nuclei reach the synaptic stage there is nothing to show whether the division is to be meiotic or mitotic, the early prophase stages being identical in both cases. Two stages in synapsis are shown in Pl. XXXII, Figs. 9 and 10. Pl. XXXII, Fig. 9, probably represents a very early stage in which the chromatic threads are just beginning to become aggregated to one side of the nuclear cavity, and are still attenuated and somewhat scattered, the appearance of the whole having much in common with that of the prophase stage shown in Pl. XXXII, Fig. 1. In Pl. XXXII, Fig. 10, the loops are shorter and the thread is thickened. The whole of the chromatic material takes part in the formation of the synaptic knot which is in contact with the nuclear membrane. In the meiotic division the membrane persists until the close of the synaptic phase.

Synapsis is followed by an extremely well-marked diakinesis stage. Where diakinesis occurs, it is usual to find all the nuclei in the sporangium in this phase, whereas the other stages more commonly tend to occur together in the same segment of the filament. This would seem to indicate that, compared with the other phases of nuclear division, diakinesis is of longer duration. Diakinesis also is the stage which occurs with the most frequency in the sporangial initials of the available material. In several cases, portions of the filament have been found which contain over 60 nuclei, all simultaneously in the diakinesis stage. The paired chromosomes assume very characteristic forms and show marked individuality, the same groupings appearing in all the nuclei (Pl. XXXII, Fig. 11).

After diakinesis, the chromosomes lengthen and are seen to be paired (Pl. XXXII, Fig. 12). The members of each pair are frequently twisted round one another as in the somatic nuclei, but here, as also in the diakinesis stage, the reduced number is clearly seen. Each of the twelve pairs consists of two whole chromosomes, and the whole group is arranged in one plane parallel to the surface of the filament.

As in the somatic division no spindle has been seen. The chromosomes move apart and become incorporated into two daughter nuclei.

In the anaphase of the heterotype division (Pl. XXXII, Fig. 13), the chromosomes have already undergone longitudinal fission in readiness for the next division, and the two halves of each chromosome form a V, the point of which is directed towards the pole to which it is passing. This early fission of the chromosomes distinguishes the anaphase of the heterotype division from that of the homotype division which follows it, as also does the fact that one group is bigger than the other although containing the same number of chromosomes. In the bigger group the individual chromosomes appear bigger, and they are more widely scattered (Pl. XXXII, Fig. 13). Polar views of the two chromosome groups from the same heterotype

anaphase are shown in Pl. XXXII, Figs. 14 *a* and *b*. This difference in size is still seen in the early stages of the interphase between the heterotype and the homotype division (Pl. XXXII, Fig. 15), but subsequently it cannot be detected, either between different nuclei or between the two halves of the same nucleus when dividing homotypically.

The homotype division, of which the anaphase is shown in Pl. XXXII, Fig. 16, is perfectly normal, and gives rise, without further division, to the spore nuclei. Each spore nucleus, together with a relatively large amount of cytoplasm and one or two pyrenoids, gives rise to a spore.

The behaviour of such spores on release has not been observed, as the material available was already fixed. The zooids released from the sporangia of *C. flavescens* at the time when the preparation in question was made acted as gametes and fused in pairs. If then the species described above is *C. flavescens* (which is probable), it would appear that the sporangia in which reduction of the chromosomes takes place are gametangia producing haploid gametes. Only a further investigation of living material, however, could decide whether these zooids fuse or whether a haploid plant bearing true gametes is formed by their germination as in other algae.

Dr. Fritsch (5), in *British Freshwater Algae* (1927), states that 'The diploid phase in the life cycle is thus, in all freshwater algae (excluding Bacillariales), restricted to the zygospore or oospore, and regarded purely from the cytological standpoint these are the only phases comparable with the sporophyte of the higher plants'. This statement, until quite recently, was thought to be applicable not only to freshwater algae but to the Chlorophyceae in general, whether freshwater or marine species. This species of *Cladophora*, however, is undoubtedly diploid, and until after the meeting of the British Association in September 1928, when the preliminary account of it was given, the author was unaware of any other work having been carried out which would cast doubt on the statement quoted. In October 1928 a paper by Schussnig appeared (2), in which he described similar results to those given above in five unnamed species of *Cladophora* from the Gulf of Naples, and mentions a previous paper of his appearing in the *Österreichische Botanische Zeitschrift* earlier in the same year, describing reduction division in *C. glomerata*. He refers also to the work of M. M. Williams in a paper published by the Linnaean Society of New South Wales as early as 1925, in which reduction division is described in the developing gametangia of *Codium tomentosum*. The case of *C. tomentosum* thus appears to be the first one described of a diploid green alga. In a second paper Schussnig (3) identifies the one species, which he described in detail in the paper already referred to, with *Cladophora Sukhriana*, a species with a haploid chromosome number of 6 or 6+1. This extra chromosome represents the sex chromosome, which he states to have been derived from the nucleolus. A second, unnamed species is referred to, this

having a haploid chromosome number of 12, i.e. the same number as that found for the species described here.

The chief interest of Schussnig's papers lies in the fact that he appears to have established the presence of a definite alternation of morphologically similar diploid and haploid generations in certain species of *Cladophora*. If this be true for all species, and if the one here described is to be identified with *C. flavesceus*, then it would appear that the gametes mentioned above, which fused, and the preparation in which the cytology is known must have come from different generations.

The principal points in which this species differs in the details of its nuclear divisions from *C. Sultriana*, described by Schussnig, are the absence of recognizable sex chromosomes, the complete disappearance of the nucleolus during nuclear division, and the absence of centrioles which Schussnig finds to be recognizable even in the resting-stage of the nucleus and in the chromosome number.

It would be of interest to compare the results given here with those of Schussnig for the other species he has examined, but of which he does not give a detailed description in either of his two preliminary papers.

SUMMARY.

All stages in the division of the vegetative nuclei of a species of *Cladophora* (probably *C. flavesceus*) have been studied. The chromosome number is 24. No continuous spireme is found in the prophase stages.

In the terminal parts of the filaments the phenomena of reduction division are manifest, the diakinesis stage being especially marked.

One homotype division, showing the reduced number of chromosomes (12), follows the heterotype division and precedes the differentiation of the spores.

The plant is thus diploid, giving rise to haploid spores following a reduction division in the terminal sporangia.

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 4. T'SERCLAES, J. de : Cytology of *Cladophora glomerata*. La Cellule, xxxii, pp. 313-26, 1922.
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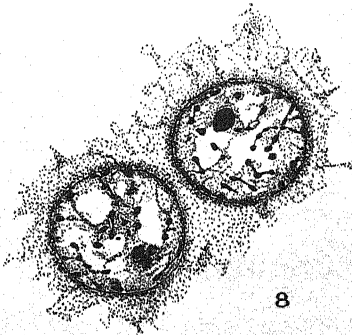
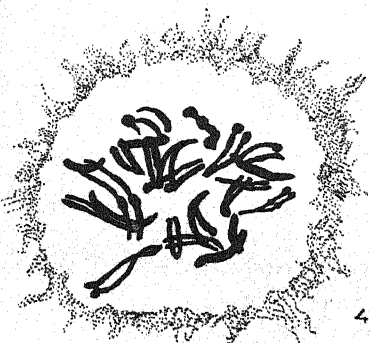
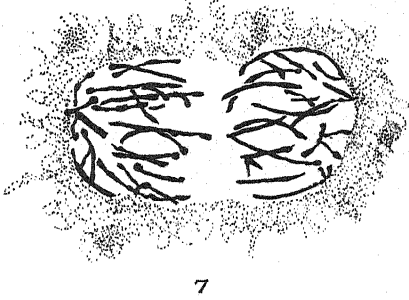
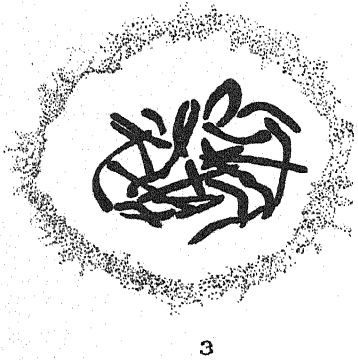
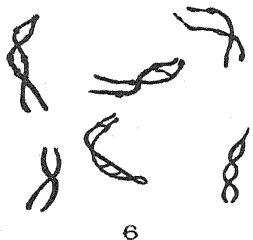
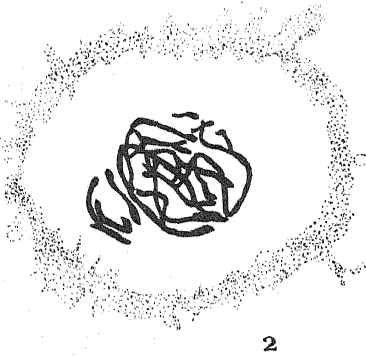
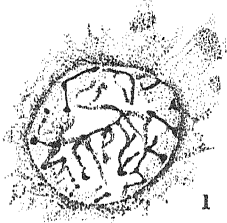
DESCRIPTION OF PLATE XXXII.

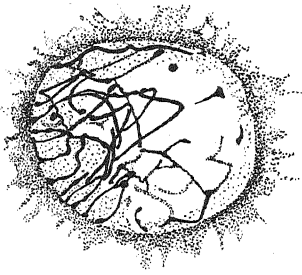
Illustrating Dr. E. Marion Higgins's paper on Reduction Division in a Species of *Cladophora*.

- Fig. 1. Prophase of mitotic division.
- Fig. 2. Late prophase. Beginning of differentiation of chromosomes.
- Fig. 3. „ „ Chromosomes fully differentiated.
- Fig. 4. Chromosomes on metaphase plate. Many show a longitudinal fission.
- Fig. 5. Chromosomes on metaphase plate. All are divided longitudinally.¹
- Fig. 6. Group of longitudinally divided chromosomes from different nuclei showing bridges of chromatic material between the two halves, and the twisting of the halves round one another.
- Fig. 7. Anaphase.
- Fig. 8. Reorganized daughter nuclei.

Meiosis.

- Fig. 9. Early synaptic stage.
- Fig. 10. Synapsis.
- Fig. 11. Diakinesis.
- Fig. 12. Paired chromosomes on metaphase plate.
- Fig. 13. Anaphase showing longitudinally divided chromosomes.
- Fig. 14. Polar view of the two halves of the same nucleus in anaphase.
- Fig. 15. Reorganization of daughter nuclei after the heterotype division.
- Fig. 16. Anaphase of the homotype division.

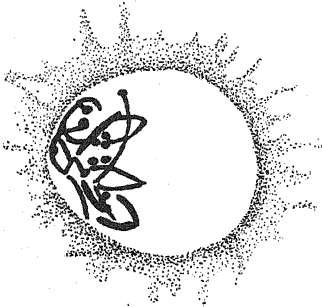




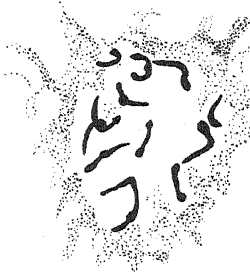
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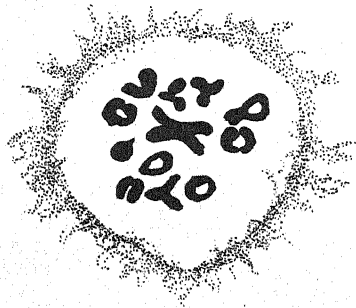


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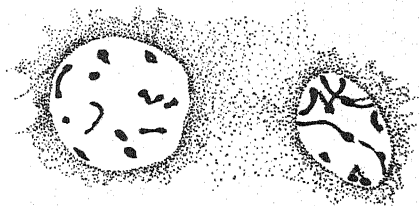


b

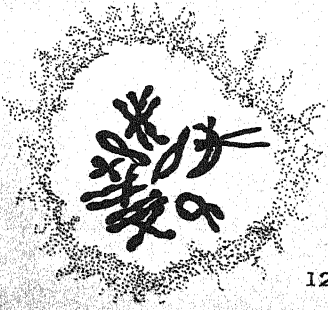
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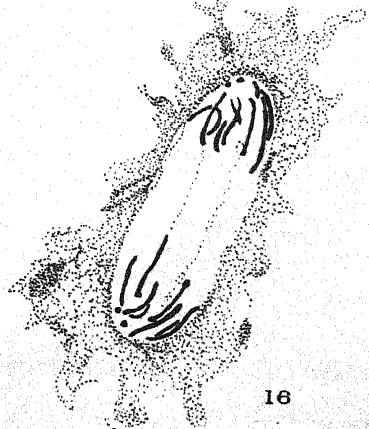
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Studies in the Gramineae.¹

IX. 1. The Nodal Plexus. 2. Amphivasal Bundles.

BY

AGNES ARBER, M.A., D.Sc.

With thirteen Figures in the Text.

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I. THE NODAL PLEXUS.

(i) *Introduction.*

IN the internodes of Grass haulms, the course of the bundles is vertical, but at the level of the nodes horizontal strands are met with, which often anastomose into an elaborate plexus. Conflicting views have been expressed by various botanists as to the origin and history of these

¹ For references to previous papers in this series, which represents the work carried out with the aid of a grant from the Dixon Fund of the University of London, see 3-10 in the Literature cited, p. 619.

horizontal strands, and it is impossible to discover from the literature what are the actual facts of the case. So, in the hope of getting further light on the subject, I have cut serial sections of nodes from the aerial shoots of various Grasses. I am indebted for material to the Director of the Royal Botanic Gardens, Kew, and to the Curator and the Superintendent of the Cambridge Botanic Garden. I have also used preparations made in conjunction with the late Ethel Sargent for our work on Grass seedlings (19 and 20). I will first describe my observations, and then discuss them in connexion with the views of previous writers. In the descriptive section, certain seedling nodes are first treated, and then more mature examples.

(ii) *Description.*

(a) *Coix lacryma-Jobi*, L.

An account of the general structure of the seedling of *Coix lacryma-Jobi*, Job's Tears, will be found in 20, p. 195. The present description and figures all relate to the region from the coleoptile insertion upwards in one seedling, which showed a well-developed plexus of horizontal strands in connexion with the crowded nodes of the first plumular leaves. This plexus is indicated in Fig. 1, B₁, p. 595. The network is not completely seen in any one section, so the horizontal strands in the diagram have been drawn from about six sections; they thus represent so much of the plexus as is found in a length of about 60 μ . A small part of the network is shown in greater detail in Fig. 1, B₂. The most striking feature is the contrast between the well-differentiated vertical strands (leaf-traces) and the horizontal bundles, which are almost wholly embryonic. The horizontal strands link up with some of the minor bundles, but these connexions are unimportant. The leaf-trace system is indeed little affected by the plexus. The median strands of the first three plumular leaves are already clearly recognizable even at so low a level as Fig. 1, A, and they pursue their course unchanged through the plexus. The two main laterals¹ of the third leaf can also be traced down to the level of Fig. 1, A, where they arise from that superficial vascular complex which also supplies the roots. One of these laterals is simply a branch of the root-girdle; the other consists of a branch of the girdle fused with a second bundle. The horizontal branch marked *minor lateral of leaf 3* in Fig. 1, A, moves part way across the axis, turns upwards, and forms the right hand of the two central bundles in Fig. 1, B₁. The left-hand member of the pair is a branch from the plexus. These two fuse higher up to form a minor lateral to the right of leaf 3. The median strand of leaf 4 is derived from a minor lateral to the left of leaf 3, marked (*l₃ + m₄*) in Fig. 1, B₁. A minor lateral for leaf 4 arises from the root-girdle just outside a main lateral for leaf 3 (Fig. 1, A). From this minor lateral of leaf 4,

¹ I use this expression here for the two laterals of the main series which are the nearest to the median strand, but are each separated from it by one or more smaller bundles.

a bud bundle, b_1 , is given off in Fig. 1, B_1 . In Fig. 1, C, five procambial bundles (b_1 – b_5) are distinguishable in the bud in the axil of the first plumular leaf; b_1 and b_2 are the two prophyll bundles, while b_3 is the median strand

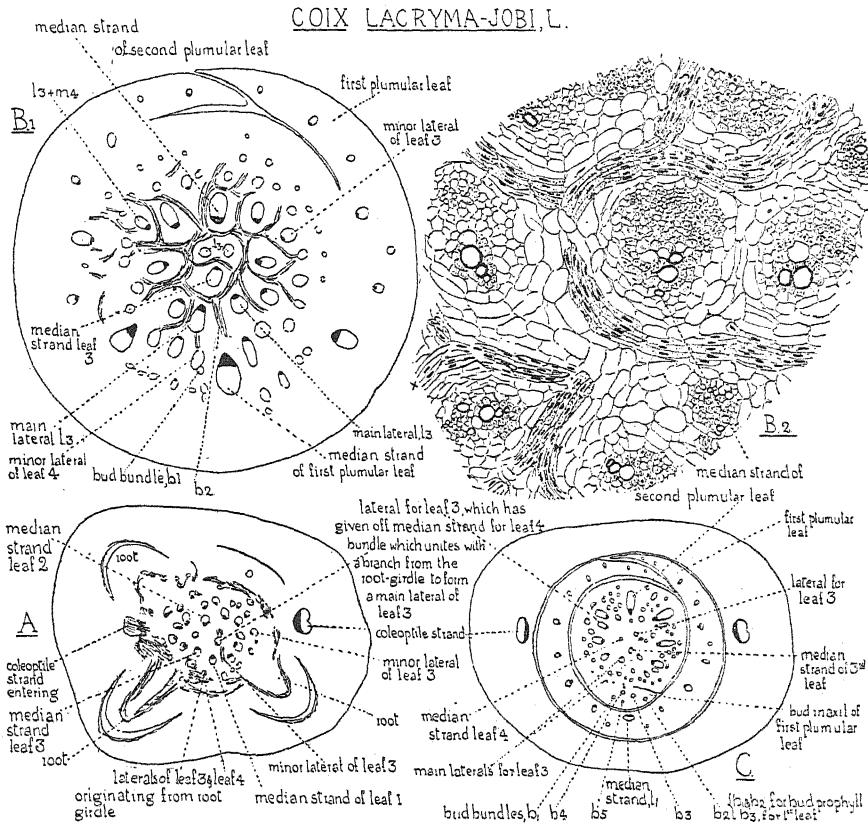


FIG. 1. *Coix lacryma-jobi*, L. Sections from a transverse series from below upwards through a seedling. A, at level of exsertion of coleoptile, and showing attachment of three adventitious roots ($\times 19$). B_1 , at the level at which the first plumular leaf is beginning to detach ($\times 39$). The nodal plexus is sketched from about six sections. B_2 , a small part of a section slightly lower than that principally used in B_1 , to show the nodal plexus in detail in the neighbourhood of the median strand of the second plumular leaf ($\times 163$). Strand \times contains a little lignified xylem; all the other horizontal strands are procambial. C, transverse section at a level between the detachment of the first and second plumular leaves, showing the bud in the axil of the first plumular leaf, not yet fully detached ($\times 19$).

for the first leaf. The origin of b_2 and b_3 cannot be traced as they are so embryonic; b_3 and b_4 are derived from the nodal plexus; b_1 , as we have already shown, takes its origin from a minor lateral of leaf 4. Since it faces b_3 (the median strand of the first leaf after the prophyll) we may take b_1 to be, in a morphological sense, the median bundle of the prophyll.

Three adventitious roots are emerging at the level at which the coleoptile is exserted (Fig. 1, A). The vascular supply of these roots has no obvious and direct connexion with the nodal plexus above them.

(b) *Avena sativa*, L.

Details of the structure of the Oat seedling below the coleoptile node will be found in 20, pp. 170 et seq. There is a network of horizontal strands in connexion with the nodes; the internodes of the plumular axis are so much abbreviated that there is scarcely an interval between the plexus associated with the coleoptile insertion and that of the succeeding node, which is shown in Fig. 2, 2. This crowding of the nodes in Grass seedlings and consequent lack of separation between the networks associated with them, sometimes results in a high degree of complexity. In the seedling of *Zizania aquatica*, L. (Fig. 10, A, p. 613), for instance, the plexus extends continuously down to the level of exsertion of the coleoptile, and up through the telescoped internodes to the level of exsertion of the fourth plumular leaf, i. e. through a distance of more than two millimetres.

In *A. sativa*, L., the median strands of the first and second plumular leaves (m_1 and m_2), all the laterals marked l_1 and l_2 , and three of those marked l_3 , can be followed right through from the plumular axis below the crowded nodes (Fig. 2, 1), to the level at which four plumular leaves have separated from it (Fig. 2, 4). The three bundles labelled m_3 in Fig. 2, 1, fuse in passing up to form the median strand of the third plumular leaf. The bundle marked l_3^* (Fig. 2, 3) arises as a branch from the horizontal plexus. In Fig. 2, 4, the median strand of leaf 4 is seen. In this young phase it has no attachment to any lower trace, and does not descend to the level of Fig. 2, 3.

The bundles marked b in Fig. 2, 3, enter the bud which arises in the axil of the first plumular leaf. One of these can be traced down to Fig. 2, 1, and farther—in fact, down to the ‘root-plate’ at the coleoptile insertion (20, Fig. 17, IV, p. 175); it remains distinguishable below the level at which the coleoptile bundles have entered the axis. Another of the bud bundles is given off by a lateral strand of leaf 2. A third, b^* , arises as a branch from the horizontal plexus.

(c) *Leersia oryzoides*, Sw.

In a previous paper the anatomy of the mesocotyl in *Leersia oryzoides*, and the relation to it of the coleoptile and scutellum bundles have been described (20, p. 183). But we are here concerned with a higher region, that of the first node. In Fig. 3, 1, p. 598, the vascular tissue forms a broken ring, in which three bundles for the first plumular leaf (m_1 , l_1 , and l'_1) and three for the second (m_2 , l_2 , and l'_2) are lignified and distinctly recognizable. In addition, there are smaller intervening masses of vascular tissue, destined for leaf 3, which in Fig. 3, 2, have differentiated themselves more clearly and have become attached to the horizontal plexus in the following way. A branch given off between m_2 and l_1 divides into two. Half becomes horizontal and forms a connexion with

the branch from the other side of l_1 , which, however, keeps its vertical course. The horizontal branch meets and unites with the end of corresponding horizontal branches from the north and south sides of l'_1 , and also with

AVENA SATIVA, L. (plumular bud, coleoptile omitted except in Fig 4)

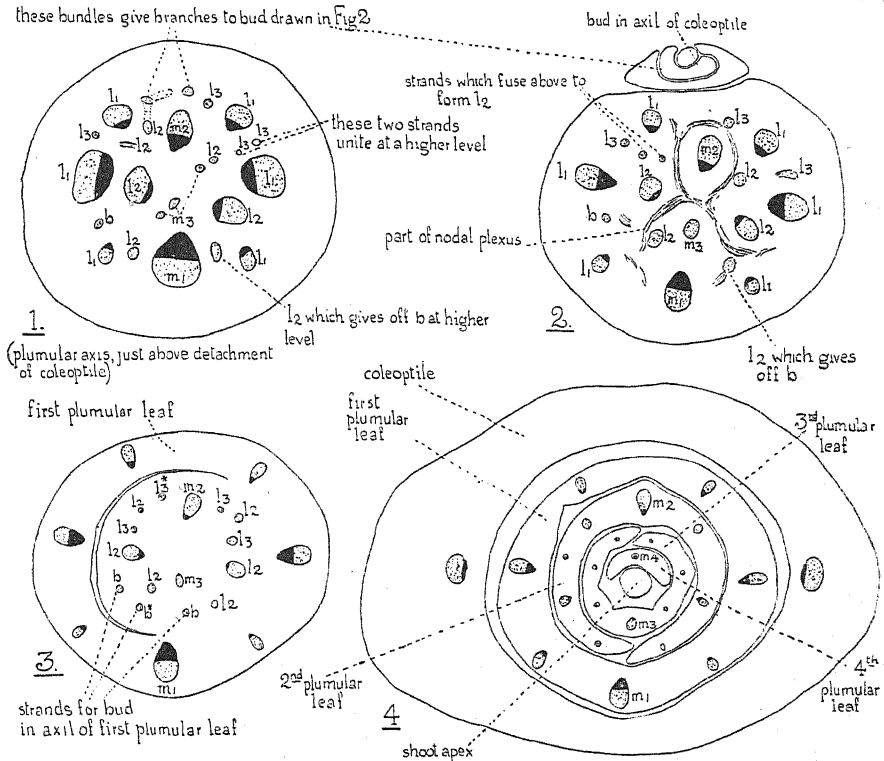


FIG. 2. *Avena sativa*, L. Transverse sections from a series from below upwards through the plumular bud ($\times 47$), from 1, plumular axis just above detachment of coleoptile, to 4, through shoot apex, traversing four plumular leaves. Coleoptile shown in 4 only; m_1, m_2, m_3, m_4 , median strands, and l_1, l_2, l_3 , laterals, for the first, second, third and fourth plumular leaves. Fig. 2, from more than one section, showing part of the nodal plexus of horizontal strands; b , bundles of bud in axil of first plumular leaf. The bundles b^* and l_3^* in Fig. 3 arise as branches from the horizontal plexus.

a branch of l'_2 . The parts of these strands which keep their vertical course unite to form l_3 on one side and l'_3 on the other, in Fig. 3, 3. The median bundle of the third leaf, m_3 , fuses with the strand to the left of l'_2 . The median strand of the fourth leaf, m_4 , is given off as a branch from the bundle l_3 .

The adventitious root seen in Fig. 3, 1, takes its origin lower down at the coleoptile insertion.

(d) *Phalaris arundinacea*, L., f. *picta*, L.

The nodal plexuses hitherto described have belonged to young seedlings. We will now consider for comparison the structure of older nodes in

two other plants, *Phalaris arundinacea*, L., *f. picta*, L., Ribbon-grass, and *Avena barbata*, Brot., a Mediterranean species of Oat.

In *P. arundinacea*, L., *f. picta*, I have made a special study of a single node—the seventh from the apex of a young leaf-bearing shoot belonging

LEERSIA ORYZOIDES, Sw.

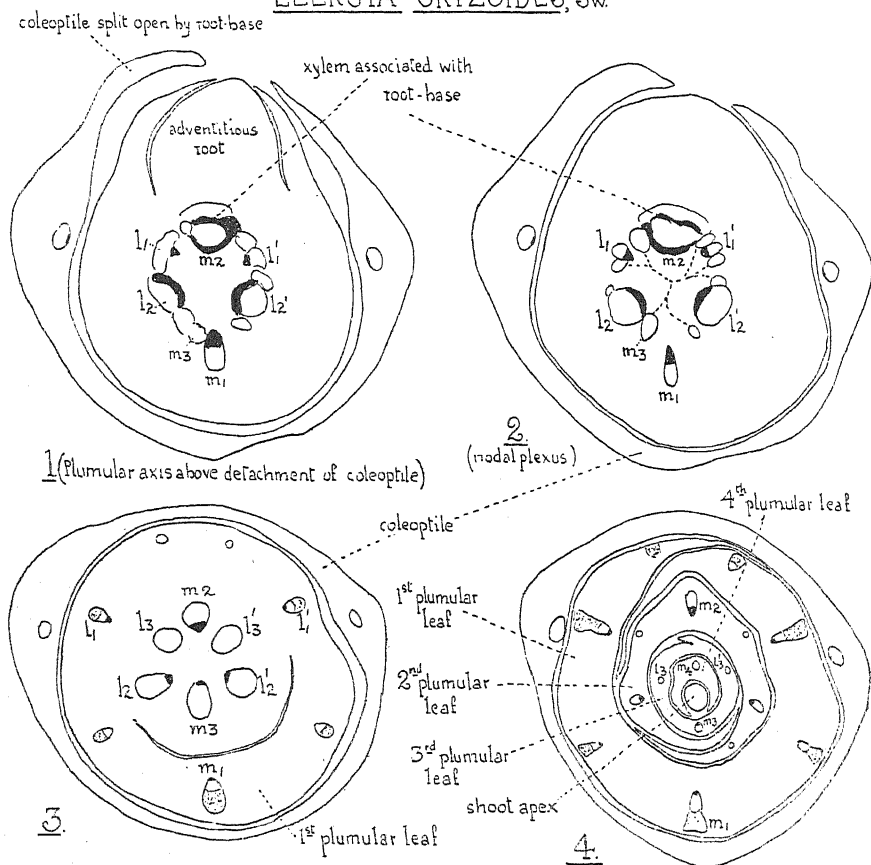


FIG. 3. *Leersia oryzoides*, Sw. Transverse sections ($\times 103$) from a series from below upwards through the plumular axis of a seedling from below the exertion of the first plumular leaf to the apex of the plumular bud. Median strands of successive plumular leaves are marked m_1, m_2, m_3, m_4 , and the lateral bundles l_1 and l'_1, l_2 and l'_2, l_3 and l'_3 .

to an adult plant. This node, owing to the extreme abbreviation of the embryonic internodes which succeed it, is only about 2 mm. from the apex. Fig. 4, I, p. 599, shows, in transverse section, the internode below the node in question. The two groups of bundles which I have chosen to follow are labelled A-F (traced in Figs. 4, p. 599, and 5, p. 601) and J-T (traced in Fig. 6, p. 603). Owing to the number of the bundles and their complex history, it was found impracticable to apply Gravis's nomenclature (16); indeed, I think that his rigid scheme may be somewhat dangerous, since it

PHALARIS ARUNDINACEA, L.

seventh node from shoot-apex
of a plant of *f. picta*, L.

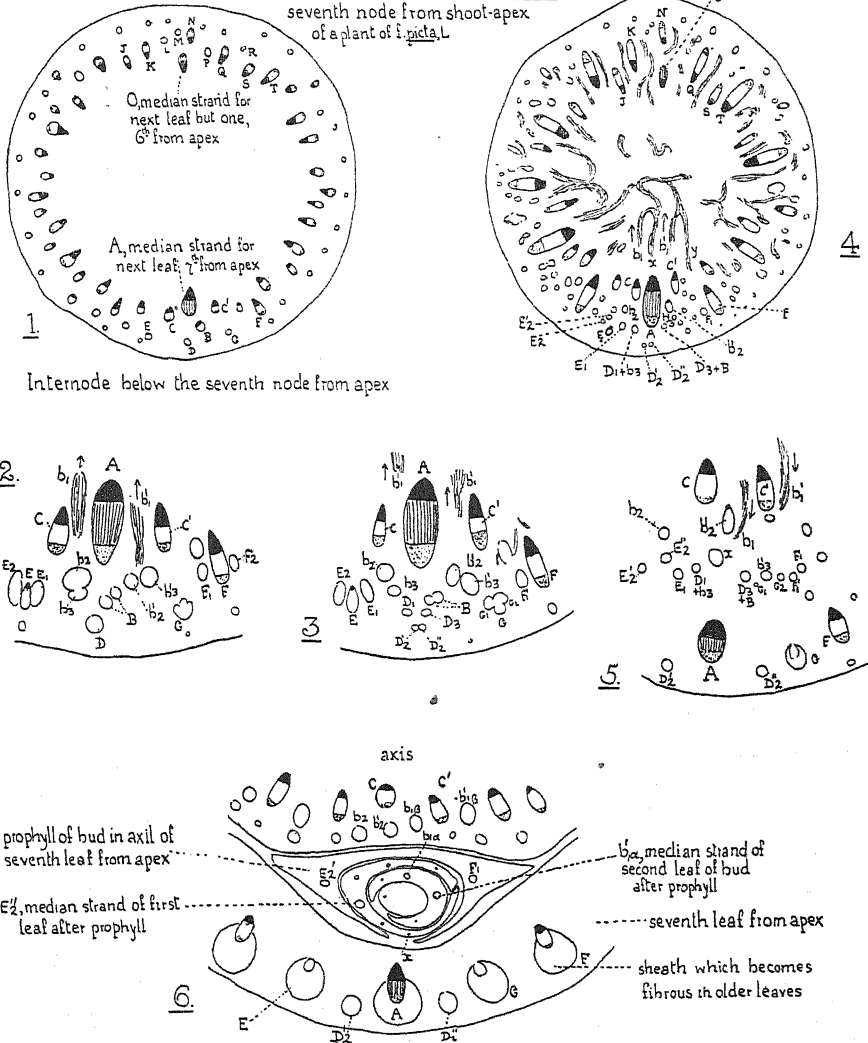


FIG. 4. *Phalaris arundinacea*, L., *f. picta*, L., Cambridge Botanic Garden, July 6. (All the diagrams in Figs. 4, 5, and 6, are drawn from one immature node, the seventh from the apex of a shoot, and the region immediately above and below it. All are from transverse sections from a series taken from below upwards. The figures are semi-diagrammatic, and no attempt has been made to record the exact size and shape of the individual bundles. For description see text, pp. 598-604). 1, internode below the seventh node from the apex ($\times 23$), A-F, group of bundles in the neighbourhood of A, the median strand of the seventh leaf from the apex; J-T, group of bundles on the opposite side of the internode; the history of these two groups is traced in the later diagrams. 2, 3 and 5, the group of bundles in the neighbourhood of A ($\times 47$), drawn at successively higher levels above 1, to show the subdivision and branching of the bundles, and the alterations in their distribution. 4, the whole node at a level between 3 and 5 ($\times 23$), showing part of the plexus of horizontal strands. 6, median part of seventh leaf from apex at a level above 5 ($\times 47$), to show the bundle relations of this leaf, its axillary bud, and the next internode.

is liable to give an impression of greater regularity than really exists. The lettering I have used is arbitrary, and attempts no significance. The bundle A will be the median bundle in the next leaf, and O, in the next leaf but one. These two bundles are shaded, to give fixed points for the eye in following the diagrams. In Fig. 4, 2, the group of bundles in the neighbourhood of A is seen at a higher level, and on a larger scale, than in Fig. 4, 1. A number of the bundles have divided; F and E have given off branches, vertical like themselves, and B has divided into two. We see here the first indication of the nodal plexus—the appearance of two horizontal branches, b_1 and b'_1 , associated with A. The exact mode of connexion between these bundles is shown in fuller detail in Fig. 5, 1 A and B, p. 601, which are below Fig. 4, 2; Fig. 5, 2, shows the mode of origin of two vertical branches from F. In both cases the connexion is established through the intra-fascicular cambium of the vertical bundles; Fig. 5, 1 A, is cut at the level of origin of b'_1 , but b_1 arose at a slightly lower level. (In these descriptions I shall for convenience speak of the horizontal bundles as *arising by branching* of the vertical ones, without prejudice to the question of whether these connexions are original or merely secondary.) From their point of origin from A, b_1 and b'_1 each develop in two directions. Each passes upwards and outwards for a short distance; they then curve towards one another and become attached on either side of the non-lignified bundle external to A, labelled B (Fig. 5, 1 B). But their main development is slightly upwards in the opposite direction, advancing towards the centre of the stem. In the course of their differentiation in this sense they form very slight attachments to the faces of C and C', which lie towards A. It would better represent the connexions of these bundles if b_1 were called $(A_1 + B_1 + C_1)$ and b'_1 , $(A'_1 + B'_1 + C'_1)$. But the subsequent history of b_1 and b'_1 is too complex for such labelling in the diagrams. A small vertical bundle, b_2 , arises from b_1 at the level of its junction with B, and a corresponding bundle, b'_2 , is developed from b'_1 . A branch, b_3 , is given off from b_2 , and b'_3 from b'_2 . If the branch b_1 is followed, it is found that after running horizontally inwards, as described, it turns vertically upwards near the centre of the axis. It then again becomes horizontal and moves outwards, passing close between C' and b'_2 (Fig. 4, 5), and then turns upwards vertically to form one of the bundles of the next internode, $b_{1\beta}$, giving off also a branch, $b_{1\alpha}$, which enters the axillary bud. In this statement I have disregarded the anastomoses which b_1 forms with other horizontal bundles; in Fig. 4, 4, it is seen in its inward passage, united with other strands. The bundle b'_1 follows a similar course. It first passes horizontally inwards, then runs vertically, and then turns outwards. Its connexion with other bundles of the horizontal plexus during its inward journey is shown in Fig. 4, 4, and during its later outward passages in Fig. 6, 5, p. 603. These two figures give some idea of the general construction of the nodal plexus. It is seen in

Fig. 6, 5, that a connexion can be traced between b'_1 and bundles as remote as L, P, Q, and T. In Figs. 4, 2, 3, and 5, certain changes in the minor bundles can be followed. In Fig. 4, 3, D is seen to have divided into three bundles, D_1 , D_2 , and D_3 ; and D_2 has again split into D'_2 and D''_2 . In

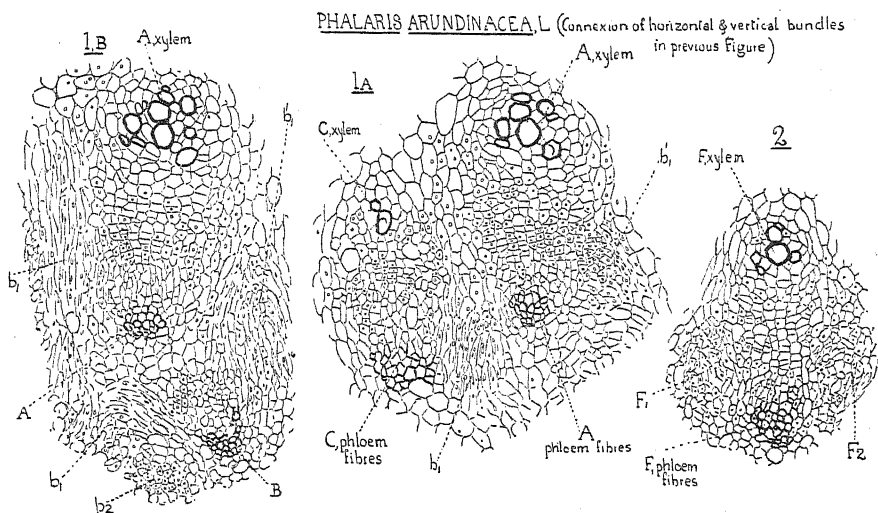


FIG. 5. *Phalaris arundinacea*, L., . *picta*, L. Transverse sections ($\times 163$) from the same node as Fig. 4, p. 599, and Fig. 6, p. 603, to illustrate the origin of horizontal branch bundles in greater detail than in Fig. 4, p. 599. 1, A (between Figs. 4, 1 and 4, 2) attachment of b'_1 to bundle A seen to the right; b_1 was attached to the cambium of A to the left, but at a lower level. 1, B, a slightly higher stage in which b_1 and b'_1 have produced outwardly directed branches fusing with B. 2 (a little below Fig. 4, 2), shows bundle F giving off strands F_1 and F_2 on either side from the cambial region.

Fig. 4, 4, fusion has taken place between D_1 and b_3 and between D_3 and B. It will be noticed in Fig. 4, 4, that the main bundles have elongated in section, and that the vertical and horizontal branching which we have described has increased the number of minor bundles, especially to the south in the neighbourhood of the midrib of the next leaf which will be axillant to a bud. In Fig. 4, 5, the minor bundles are seen arranging themselves in preparation for this bud. At the same time there has been a redistribution of the major bundles, so that A, and the other strands for the next leaf, are now nearer to the margin, while those which are related to younger leaves are further in. The row of small bundles, including E'_2 , E''_2 , E_1 , ($D_1 + b_3$), x , ($D_3 + B$), b'_3 , G_2 , F'_1 , and F_1 , will all enter the bud.

On comparing Fig. 4, 1, and Fig. 4, 6, we realize the fate of the group of lettered bundles to the south. A enters the next leaf as its median strand, F as one of its main laterals, G as one of the minor laterals, and two branches of D as minor peripheral bundles. C and C' pass up into the next internode in addition to b'_2 , b_2 , b_{1B} and b'_{1B} , which are branch strands with which A, B, C, and C' were originally involved, but which became associated with other strands in the horizontal plexus. C' enters

the overlapping margin of the next leaf (sixth from the apex), but C passes up the axis and enters the succeeding leaf (fifth from the apex).

The bud supply is surprisingly complex in its origin. One bundle for the prophyll is a branch of F, while E divides into two branches, E_1 and E_2 , one of which, E_2 , again divides, half (E'_2) forming the second prophyll bundle, and the other half (E''_2) forming the median strand of the first leaf after the prophyll, while x appears to form one of its laterals. The second leaf after the prophyll receives its median bundle from b'_1 , and the larger of its laterals from b_1 —that is to say, it receives strands whose ultimate source is A, B, C, and C'. I have not been able to determine the exact fate of the following small bundles: E_1 , ($D_1 + \delta_3$), ($D_3 + B$), δ'_3 , G_1 , G_2 , and F'_1 ; I have traced them to the base of the bud, but they are so embryonic that I cannot follow them further with certainty.

We may now leave the bundles (to the south of the diagrams) associated with the midrib of the seventh leaf from the apex, and consider the group of bundles to the north, one of which, O, is the median strand for the sixth leaf from the apex. The general relations of these bundles can be understood from Fig. 4, 1 and 4, and their history followed in detail in Fig. 6, p. 603. Fig. 6, 1, shows this group of bundles at a level above Fig. 4, 1; and Fig. 6, 2, at a level a little below Fig. 4, 4. Among the earliest events shown are the branching of N on one side and its attachment to M on the other side. K gives off a vertical bundle, K_1 , on one side, and on the other a horizontal branch, K'_1 , which forms one strand with L_1 , the branch to the left of L. T gives off two vertical branches, T_1 and T'_1 , and v gives off two horizontal branches. In the next diagram (Fig. 6, 3), which is just above Fig. 1, 4, branches from the sides of Q have become attached to branches from P and R, forming strands which may be called ($P_1 + Q_1$) and ($R_1 + Q'_1$). The branches from K, L, and N are passing horizontally inwards. In Fig. 6, 4 (which comes between Fig. 4, 4, and Fig. 4, 5, p. 599), the horizontal strands have become a more conspicuous feature. A little above Fig. 6, 4, ($R_1 + Q'_1$) connects with ($K'_1 + L_1$), and the north-west end of this compound strand fuses with the bundle ($K'_1 + L_1 + L'_1$) to the left of O. Also above Fig. 6, 4, the bundles L'_1 and ($P_1 + Q_1$) unite, and the combined strands turn up vertically; then they separate again as two horizontal bundles in Fig. 6, 5. The horizontal branch U_1 from U (seen in Fig. 6, 4) connects at a slightly higher level, near the stem surface, with T'_1 ; it dies out after forming a delicate connexion near the middle of the stem with a bundle which is the upturned vertical end of a horizontal strand entering from the north-west. This bundle originates as a branch from one of the vertical bundles of the internode. After U has died out, the arc $T_1 T'_1$, which has broken up into two lateral masses, gives a horizontal inward branch from T'_1 . This fuses with ($L'_1 + P_1 + Q_1$) and anastomoses with other bundles. On the other side of T the lateral mass divides into two branches,

PHALARIS ARUNDINACEA, L.
(seventh node from shoot-apex, plant of *f. picta*, L.)

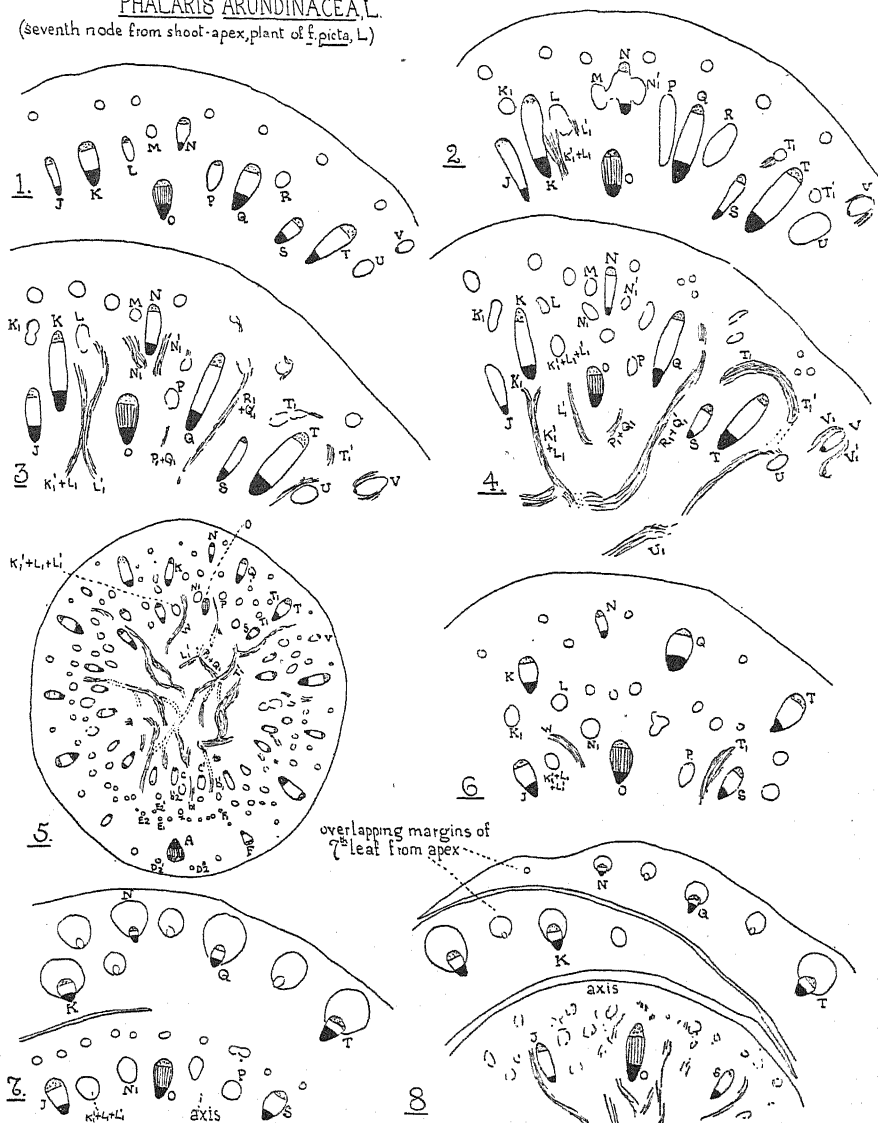


FIG. 6. *Phalaris arundinacea*, L., *f. picta*, L. The same node as that illustrated in Fig. 4, p. 599, and Fig. 5, p. 601. All drawings from transverse serial sections $\times 47$ except 5, which is $\times 23$. The diagrams illustrate the history of the group of bundles to the north in Fig. 4, 1, which are associated with O, the median strand for the next leaf but one. As in Fig. 4, A and O, the median strands for the seventh and sixth leaf from the apex are shaded to give a fixed point in the diagrams. For description see text, pp. 603-4. 1, internode below seventh node from apex, between Figs 4, 1 and 2. 2, just below Fig. 4, 1. 3, just above Fig. 4, 1. 4, between Figs. 4, 1 and 4, 5. 5, just above Fig. 4, 5; general view on a smaller scale to show part of the plexus of horizontal strands. Connexions occurring not exactly at this level are dotted. 6, between Figs. 4, 5 and 4, 7. 7, just below Fig. 4, 6; the seventh leaf from the apex is just beginning to detach. 8, above Fig. 4, 6: the leaf is now free and its margins have separated.

each marked T_1 , and one of them passes inwards at a higher level. Fig. 6, 5, which is just above Fig. 4, 5, shows the whole section on a smaller scale, and gives a general view of the plexus of horizontal bundles. That marked W

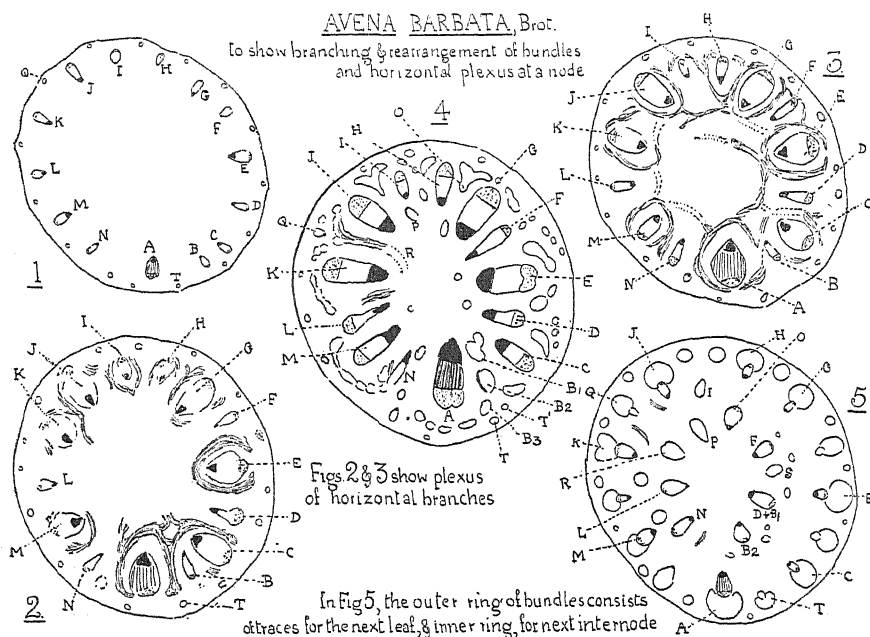


FIG. 7. *Avena barbata*, Brot. Transverse sections from a series through a young node ($\times 23$). The median bundle, A, of the leaf exerted at the node is shaded. For explanation see text, pp. 605, 606. 1, internode below node studied. 2, appearance of horizontal branches from the ring of bundles. For mode of attachment of vertical and horizontal branches see Fig. 8, p. 605, in which the history of bundle E is followed. 3, slightly higher than 2, to show horizontal plexus; the branches not visible exactly at this level are dotted. 4, further history above the plexus. 5, just below detachment of leaf; the inner bundles form a ring for the next internode.

is derived from K and L. The dotted lines show connexions which do not occur exactly at this level.

The further distribution of the northerly group of bundles can be followed in Fig. 6, 6–8. In Fig. 6, 7 (which is just below Fig. 4, 6) the leaf whose median bundle is A is beginning to detach itself from the axis. At the base it forms a closed sheath, but in Fig. 6, 8, the sheath has opened on the side remote from A, and we see that the bundle K enters the inner margin, while N, Q, and T enter the overlapping margin. The strands O, J, and S form major bundles for the next internode; one of the minor bundles of the same ring (that to the left of O, the median bundle) is formed by a branch of N, while that between it and J consists of branches K and L (Fig. 6, 7); branches from K and L also take part in the formation of the ring of very small strands placed closer to the periphery. In Fig. 6, 8, the formation of horizontal strands in connexion with the next node has already begun.

(e) *Avena barbata*, Brot.

In *A. barbata* I have studied a single node from a mature plant. I have not got the shoot apex in my series, but I believe, from internal evidence, that the shoot was a young inflorescence axis; the leaf borne at

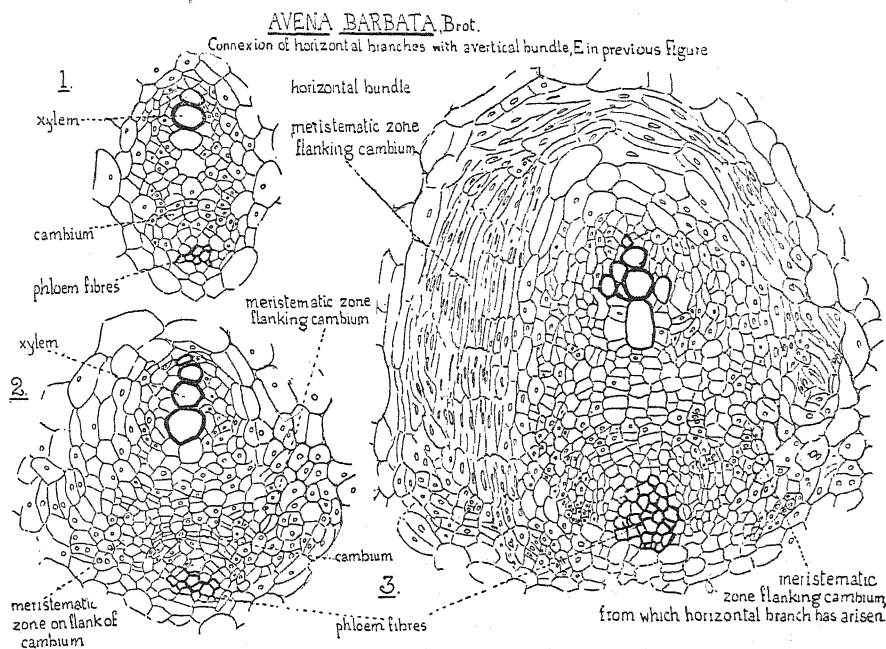


FIG. 8. *Avena barbata*, Brot. Transverse sections from a series through the bundle E in Fig. 7, p. 604, to show the connexion of the horizontal branches with the meristem flanking the cambium ($\times 163$). (Orientation not uniform with Fig. 7.) 1, bundle E in Fig. 7, 1; 2, bundle E between Fig. 7, 1 and 2; 3, bundle E in Fig. 7, 2.

the node in question had no axillary bud, so this node has the advantage of enabling one to examine a plexus of horizontal bundles which has no connexion with any bud (or root). Fig. 7, 1, p. 604, shows the internode below this node. For the sake of having a fixed point to follow in the diagrams, the median bundle for the next leaf exerted is shaded; the lettering is arbitrary. In Fig. 7, 2, horizontal branches are appearing between the bundles, and forming arcs around their inner apices. It is difficult to give an exact idea of the relations of these branches in a diagrammatic sketch, so, in Fig. 8, above, I have drawn the bundle E at three successive levels on a larger scale. Fig. 8, 1, shows the bundle at the level of Fig. 7, 1; it has a small but clearly distinguishable cambium. In Fig. 8, 2, which is a little higher, meristematic activity has invaded the parenchyma on either side of the cambium. Fig. 8, 3, which is from the same section as Fig. 7, 2, shows a further stage, in which this meristematic activity has resulted in the formation of a horizontal arc of procambium,

enclosing the protoxylem. In Fig. 7, 3, an attempt is made to show the complexity of the network of horizontal strands which become connected right round the inner faces of the bundles, and which also branch and interlace in a way that defies exact tracing out. From this horizontal plexus a number of new vertical bundles arise. The origin of the following bundles seen in Fig. 7, 4 and 5, has been definitely traced from the horizontal plexus: R from between J and K; O from between G and H; S from just inside E; while P is also traceable to the plexus. I have followed up the two minor bundles T and Q (Fig. 7, 1), and I find that neither takes any part in the plexus, but that they both continue straight up into the next leaf (Fig. 7, 5). In Fig. 7, 5, I has passed somewhat inwards, but this is because it enters the inner of the overlapping margins of the next leaf. Of the ring of bundles in Fig. 7, 1, those that will not enter the next leaf, but will pass on into the succeeding internode, are N, L, F, D and B.

(iii) Discussion.

(a) The Vascular Relations of the Adventitious Roots.

I have not studied in any detail the attachment of the adventitious roots to the parent axis at the node; but those roots met with incidentally while examining the nodal plexus (*Coix lacryma-Jobi*, L., Fig. 1, A, p. 595; *Zizania aquatica*, L., Fig. 10, p. 613; *Leersia oryzoides*, Sw.; and *Phalaris arundinacea*, L.) agree in the fact that their vascular tissue is attached to the superficial layers of the stem cylinder which may be differentiated to form a definite 'root-girdle', e.g. *Z. aquatica*. In *Zizania* the inextricable tangle of horizontal bundles, in and about the region of the first node, is closely associated with the root-girdle.

In the less vascular seedlings of *Coix* and *Leersia* studied, anastomoses may no doubt be traced between the superficial root-supply layers of the stele and the nodal plexus, but these connexions are merely subsidiary. In *L. oryzoides*, for instance, one extremely rudimentary root is exserted at the first node, but though it is clearly connected with a delicate root-girdle which surrounds the stele it has, at this young stage, no obvious relation to the nodal plexus which is already in existence. And in an adult plant of *P. arundinacea*, an embryonic root-girdle, with which the two young roots were associated, surrounded the stele of a node which showed a bundle plexus, but there was no apparent connexion between the girdle and the plexus. Moreover, in *C. lacryma-Jobi*, in which three well-developed roots were seen arising at the exsertion of the coleoptile (Fig. 1, A, p. 595), the main development of the nodal plexus did not occur at the level of origin of the roots, but distinctly higher.

Though these observations are merely cursory, they indicate that the nodal plexus does not *originate*, as has sometimes been suggested, through

the entry of strands from the roots, though these strands no doubt become connected with it secondarily. Such a conclusion is consistent with the views of von Mohl, who long ago emphasized the essential independence of the vascular system of stem and adventitious root (18). Moreover, as Bugnon (14) has pointed out, a nodal plexus may exist in rootless nodes, so that the proposition that it originates from the root strands could, at the best, have no general validity.

(b) *The Vascular Relations of the Buds.*

I have described the sources of the bundles in a bud in the axil of the first plumular leaf of a seedling of *Coix lacryma-Jobi*, L., and of *Avena sativa*, L., and in the axil of a later foliage leaf belonging to the shoot of a mature plant of *Phalaris arundinacea*, L. The facts of the vascular supply in these cases may be summarized as follows:

In *C. lacryma-Jobi*, L., the bud in the axil of the first plumular leaf was very young, but five bundles (b_1 - b_5 , Fig. 1, C, p. 595) were visible in it. Two of them were too little developed to be traceable below the bud; of the other three, two arose from the nodal plexus, and one from a lateral bundle of the first plumular leaf.

In *A. sativa*, L., in the young stage examined, only three bundles entered the corresponding bud (Fig. 2, 3, &c., p. 597). One of these could be followed right up from the root-plate at the coleoptile insertion; it was distinguishable below the level at which the coleoptile bundles enter the axis. It thus had the independent, leaf-trace-like existence to which Gravis (16) and Bugnon (13) have already drawn attention for certain lateral-branch-traces (see also 7, p. 400). Another bundle arose as a branch from a lateral of the second leaf, and a third arose from the horizontal plexus.

In *P. arundinacea*, L., *f. picta*, L., the bud in the axil of the seventh leaf from the apex of a shoot (Fig. 4, 6, p. 599) received contributions from at least seven leaf-traces; these were chiefly the strands for the axillant leaf, but included also a strand destined for the next leaf and a strand for the next leaf but one.

One cannot but be struck by one feature which is common to these three buds—the irregular, one might almost say fortuitous, fashion in which the vascular supply of the bud is related to that of the parent shoot. The young bud of *Coix lacryma-Jobi*, L., showed no connexion with the bundles of its own axillant leaf, or the next leaf, but one of the five traces was attached to a bundle belonging to the third leaf. In *Avena sativa*, again, there was no connexion with the axillant leaf, but here one trace came from a lateral bundle belonging to the succeeding leaf. And in the older bud of *Phalaris arundinacea*, although a number of connexions were found with the axillant leaf, there were also connexions with the two succeeding leaves.

And it is not only in the source of the bundles, but in their arrangement within the bud, that we find an irregularity which suggests the conclusion that the attachment of the various strands to the shoot bundles is simply a matter of topography. In *P. arundinacea* the two first leaf-traces of the bud—the prophyll bundles—are laterally placed, as is usual in Grasses, and they draw their supplies from two bundles which are some little distance on either side of the median strand of the axillant leaf; one of these bundles (F in Fig. 4, 1, &c., p. 599) is much larger than the other (E), and it gives rise to that bundle of the prophyll which faces the ‘median’ strand of the succeeding leaf, and hence may be considered, in a morphological sense, as the ‘median’ bundle of the two-bundled prophyll. The first leaf after the prophyll is asymmetrical; its principal bundle, which is not accurately median in position, is derived from the same bundle, E, as the second prophyll bundle. The second leaf after the prophyll is again unsymmetrical in its vascular scheme, having, in addition to its ‘median’ strand, which is by no means central, one large and one small lateral on one side, and only one small lateral on the other. The two larger strands are ultimately derived from branches of the median bundle of the axillant leaf, which for part of their course ran horizontally in the plexus, and became linked up even with strands belonging to the other side of the internode. The relatively median position of these two branches, when they return towards the shoot surface, makes it natural that they should supply this particular leaf, which is in the heart of the bud.

The asymmetry of the two leaves succeeding the prophyll is of interest because it seems natural to suppose that it may be a parallel phenomenon to the lack of symmetry about the midrib which characterizes Monocotyledonous prophylls. When a lateral bud has grown out into a branch, its apex is supplied by its own shoot bundles, which develop without constraint, and can arrange themselves symmetrically, and hence can supply a symmetrical skeleton for the leaves. But in the young bud of *Phalaris* described, the bundles for the first three leaves are all directly traceable to the parent axis; the lateral branch-axis can scarcely be said to exist at this early stage. The bud is thus receiving its vascular system direct from a dorsiventral source—an irregular row of bundles in the parent axis on its flank (Fig. 4, 5, p. 599). It is not surprising that this should result in irregularity in the vascular supply of the bud.

The relation of the bud bundles to the nodal plexus will be considered in (d), p. 609.

(c) *Discontinuity within the Leaf- and Bud-traces.*

A question of some theoretical importance, which arises out of the study of my sections, relates to the continuity or discontinuity of the leaf- and bud-traces in their early stages. It is commonly said that the traces

of leaves and of buds make their appearance first in the base of the lateral member, and that there is then a downward differentiation linking up the vascular skeleton of the lateral appendage with that of the parent shoot. One would like to know whether this can be depended upon as a general statement, and especially whether it is applicable to all Grasses. Something of the kind is undoubtedly true of some traces. For example, the median strand of leaf 4 in *Avena sativa*, L. (Fig. 3, 4, p. 598), has at this stage no attachment to any lower trace, and no longer exists at the lower level of Fig. 3, 3. And in *Coix lacryma-Jobi*, L., two of the bud-strands (b_2 and b_3 in Fig. 1, C, p. 595) die out at the base of the bud when traced downwards. But in other traces which I have followed it is difficult to imagine that discontinuity of this type has ever occurred. In the node of *Phalaris arundinacea* described, seven of the smaller strands seen in Fig. 4, 5, p. 599, can be traced continuously up to the base of the bud and yet they are too embryonic to be followed to their destinations in the leaves of the bud (p. 602); it seems as though differentiation here were proceeding entirely upwards from below. And, returning to our seedling of *C. lacryma-Jobi*, not only the median strands of the first, second, and third plumular leaves, but even certain laterals of leaves 3 and 4, can be followed up as individuals from the level of exertion of the coleoptile strands (Fig. 1, A, p. 595) into their respective leaves. In such examples it seems improbable that each of these traces ever existed in the form of two segments, and that the gaps between these segments were then each neatly bridged. But further comparative evidence is needed before this question, which has been a subject of discussion from the days of von Mohl (18), can be treated as settled.

(d) *The Origin and History of the Nodal Plexus.*

Four divergent views have been held by botanists who have discussed the origin of the nodal plexus in Grasses. De Bary (11) and van Tieghem (22) attributed its whole organization to the bundles of the axillary bud, with which Strasburger (21) would also associate those of the adventitious roots; Guillaud (17) considered that it was entirely cauline, and bore no relation to the leaves; Chrysler (15) speaks of it as resulting from the anastomosis of the leaf-traces; and in more recent times Bugnon (12 and 14), after reviewing earlier work, has suggested that it arises by change of direction of leaf-traces which can find no room to pursue a vertical course when they enter the node from the leaf. The subject is, indeed, one of difficulty, which is mirrored in this divergence of opinion. I do not think that it is possible to come to any final solution of the problems of nodal anatomy without the use of serial sections, and it is thus not worth while to discuss the earlier work in detail; reference may be made to von Mohl (18) and the literature which he cites. The only research on the subject based on paraffin sections is that of Bugnon, who, in a seedling of *Oryza sativa*, L.,

described the first two horizontal bundles of the nodal plexus as leaf-traces, which, entering the axis at a level at which it was already stocked with bundles, found their vertical passage impeded, and were obliged by spatial considerations to take a horizontal course. It must not be overlooked that the phenomenon which he observed might be expressed in the reverse sense; the leaf-traces, which he is considering, would then be described as arising as vertical branches from the horizontal nodal plexus. But, even if his mode of description be accepted, I find it impossible to endorse the far-reaching deduction which he draws from the behaviour of these two bundles. He concludes, in general, that the transverse bundles unrelated to axillary buds in the nodes of Grasses are formed by the abrupt change of direction of the longitudinal leaf-traces. Moreover, he arrives at the further deduction, that no morphological theory depending on the course of leaf-traces can have any validity, and instances the view concerning the structure of the Grass embryo, put forward by Ethel Sargant and myself (20), as standing condemned on this ground. Since Bugnon bases so considerable a superstructure upon his idea as to the origin of the nodal plexus, it is necessary to examine it carefully. Now it seems to me that his account of the horizontal traces which he observed in *O. sativa*, though correctly describing the facts for certain bundles, is not adequate as an account of the origin of the nodal plexus *in general*. In order to substantiate this criticism, and to see what positive results can be arrived at as to the origin of the plexus, we may summarize the relevant points in the descriptive section, omitting the question of the adventitious roots, which has been already discussed (p. 606).

In *Coix lacryma-Jobi* (Fig. 1, p. 595) it is not possible to describe the plexus as originating by branching of the leaf-traces, or by their change of direction, or by the entry of bundles from the bud, since, in the seedling studied, only two of the bud-bundles were connected with the nodal plexus. The impression left on one's mind by examination of the sections is that the nodal plexus originates through an outburst of meristematic activity, which affects most of the zones of ground tissue between the bundles (Fig. 1, B 2, p. 595).

In *Avena sativa*, L., in the young stage examined, only one bud-bundle was connected with the nodal plexus, which was also markedly independent of the leaf-trace bundles (Fig. 2, 2, p. 597). Seven traces for leaf 1, seven for leaf 2, and four for leaf 3 pass unchanged through the plexus associated with the telescoped nodes, but one trace for leaf 3 arises as a branch from the plexus. Such connexions as are found between the plexus and the minor bundles are unimportant.

In *Leersia oryzoides*, Sw., we have a closer connexion between the plexus and the leaf-traces than in the two species just described. The bundles destined for the first and second plumular leaves run their course regardless of the plexus. Those for the third plumular leaf are closely associated with

the plexus, which might indeed be described as consisting of horizontal bundles proceeding from the traces of the third leaf (Fig. 3, 2, p. 598). But this fact lends no support to Bugnon's interpretation, since these traces themselves maintain their vertical direction, and are recognizable *below as well as above* the plexus.

We may conclude that, in the three species whose seedlings have been studied, the plexus is, to a marked extent, independent of the leaf-traces.

When we come to *Phalaris arundinacea*, L., and *Avena barbata*, Brot., the situation changes because we are here dealing with maturer shoots. Chrysler (15), who studied the nodes of these two species, does not discuss the origin of the horizontal plexus; in both cases he merely refers to the occurrence of anastomoses between the leaf-trace bundles. In *P. arundinacea*, at the stage which I examined, it would be natural to describe the horizontal strands forming the nodal plexus as branches of the vertical internodal bundles with which they are connected through the intrafascicular cambium. A study of the younger nodes of the same shoot shows that the horizontal bundles are from the first closely associated with the vertical leaf-traces, and that the outburst of meristematic activity in the interfascicular ground tissue which produces the horizontal strands, simultaneously affects the intrafascicular cambium of the bundles, so that connexions, such as those shown in Fig. 5, p. 601, may be established contemporaneously with the earliest appearance of the horizontal branches. These strands in the first place run radially inwards, and they form complex anastomoses. The two strands associated with the median bundle (b_1 and b'_1 , Fig. 4, 2, p. 599) were found to change their horizontal course for a vertical one near the middle of the stem, and then to run horizontally outwards again, returning to a position adjacent to the two bundles originally accompanying the median strand, but not reaching the median strand itself (Fig. 4, 5, p. 599). These bundles (b_1 and b'_1) are destined to be supply strands for the axillary bud and for the next internode. On the opposite side of the axis, where I also followed the bundles, I have not observed anything corresponding to this definite looping in and out of the horizontal strands, and I take it that it is a phenomenon specially connected with the bud. Though the bud-bundles participate in the plexus, they are not so important as to justify the idea that the plexus is their derivative.

In *Avena barbata*, Brot., the nodal plexus is formed of horizontal bundles which encircle the leaf-trace bundles in a striking fashion (Fig. 7, 3, p. 604. and Fig. 8, 3, p. 605). The horizontal strands form connexions with the principal bundles which will enter the next leaf, and also with those minor bundles which will remain in the shoot and supply the next internode. These connexions are established through a meristem flanking the intrafascicular cambium of the vertical bundles (Fig. 8, p. 605). The horizontal strands become connected into a complex network, and from this network

a certain number of vertical branches arise: in the particular case described, four of these vertical bundles enter the next internode. No buds are associated with the node illustrated, so that there can be no question here of the plexus originating from the vascular skeleton of a bud.

The results thus summarized for these five species lead to the conclusion that the nodal plexus does not depend in its origin either upon the vascular supply for the buds, or upon the change of direction of the vertical leaf-traces; we have already shown that the adventitious roots are not responsible for its inception (p. 606). I think that the evidence points to the plexus originating through the meristematic activity of the ground tissue between the vertical bundles—a recrudescence of the earlier phase of activity which initiated the leaf-traces. The connexions established between the horizontal and vertical bundles may, however, arise so early that the horizontal bundles may equally well be described as branches of the leaf-traces. The subject is altogether one of peculiar difficulty, and there is need of a fuller comparative investigation of the ontogeny of nodal structure in the Grasses than I have attempted in this paper.

2. AMPHIVASAL BUNDLES.

Amphivasal bundles are not uncommon in the nodal regions of Grasses. They are said to be of two types—one type being that in which an ordinary collateral leaf-trace is amphivasal during part of its course, and a second type, that in which the amphivasal structure is due to bundle fusions (15). We will consider the two types in turn.

To illustrate the first type, I have drawn in Fig. 9, p. 613, a leaf-trace bundle of the Bamboo, *Arundinaria spathiflora*, Trin., at three different levels to show how it changes from collateral to amphivasal, and then back to collateral in traversing its node of exsertion. The leaf which it enters is supplied with seven such strands, each of which passes through a more or less completely amphivasal phase.

Certain leaf-traces in the seedling of *Zizania aquatica*, L., were also found to be amphivasal. Fig. 10, A, shows the plumular axis between the detachment of the first and second leaf. In the centre, the leaf-traces and the plexus of horizontal bundles are encircled by a conspicuous root-girdle. The median bundle of the first plumular leaf, which is seen in Fig. 10, A, enmeshed in the plexus, is at this level amphivasal. It is shown on a larger scale in Fig. 10, B₁. At a level less than a millimeter above B₁, it has become collateral (B₂).

In examples such as those just described there is no doubt as to the amphivasal character, but I feel uncertain about some of the cases recorded in the literature. In *Avena barbata*, Brot., in such sections as Fig. 7, 2 and 3, p. 604, it will be noticed that there is a space between the vertical bundles and the intervening horizontal strands. This space in an older node

ARUNDINARIA SPATHIFLORA, Trin.
(Leaf trace passing through a node)

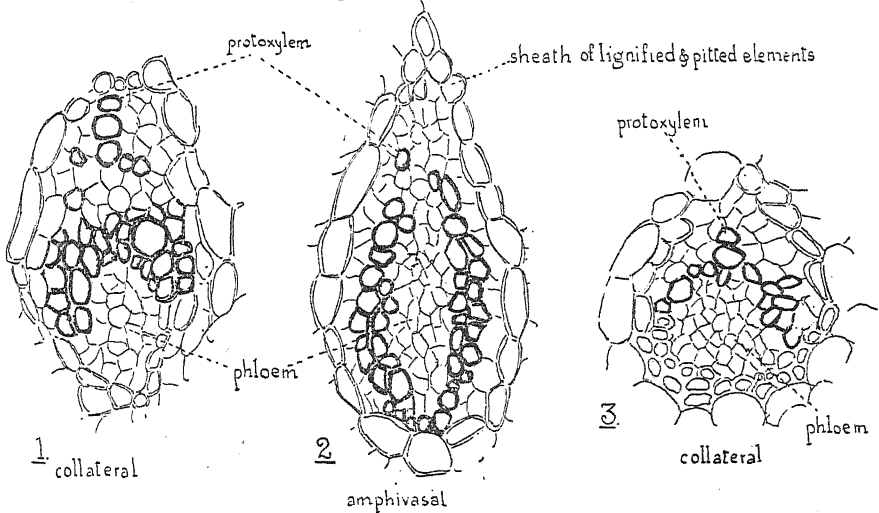


FIG. 9. *Arundinaria spathiflora*, Trin. Transverse sections ($\times 424$) from a series passing upwards from below through a node, showing the phases passed through by the median bundle of the leaf exerted at this node. Amphivasal structure in 2; collateral above and below. In the sheath of lignified pitted elements, which is specially conspicuous in 2, many of the elements are quite short.

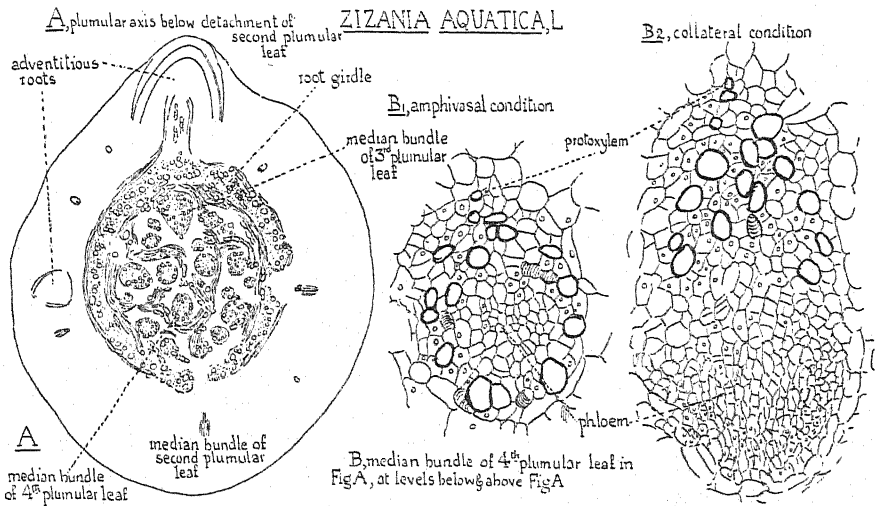


FIG. 10. *Zizania aquatica*, L. A, transverse section of plumular axis between the detachment of the first and second leaf. Semi-diagrammatic: only the lignified xylem elements, and those approaching lignification, indicated individually ($\times 23$). In the region of A, some of the bundles have a definitely amphivasal structure. One of these, the median bundle of the fourth plumular leaf, is shown in B₁, at a level a little below A; B₂, the same bundle above A; there is less than a millimetre between B₁ and B₂. B₁ and B₂ ($\times 193$).

(collected October 23) was found to be occupied by a lateral belt of pitted and reticulate thick-walled elements, developed on either side of each vertical bundle. A condition similar to that photographed for this species by Chrysler (15, Pl. I, Fig. 2) is thus produced, but I doubt whether the term 'amphivasal', which he uses for these bundles, is justified. The phloem is not completely enclosed by the pitted tissue, which, moreover, is different in character from the xylem, and seems better described as a fibrous sheath.

Chrysler figures some undoubtedly concentric bundles from the node of *P. arundinacea* (15, Pl. I, Fig. 4). The node of this species, which I have illustrated in Figs. 4, 5, and 6, was very young, and did not show bundles of this type, but I have seen them in older material (collected October 12). I have not traced their origin, but their position suggests that they are, as Chrysler claims, branches of the nodal plexus.

I have elsewhere (9) described the occurrence of five amphivasal bundles in the lemma (flowering glume) of *Hordeum trifurcatum*, Jacq., before it becomes free from the base of the spikelet. In 9, Fig. 6, p. 523, the median strand is shown in its lower concentric phase, and in its collateral phase in the free part of the lemma. Amphivasal strands are also to be found in the upper part of the coleoptile of *Avena sativa*, L. Outside the Gramineae, I have recorded the existence of similar concentric bundles in the leaves of several species of *Triglochin* (2, pp. 57–8 and Pl. VIII, Fig. 6, C, and Pl. IX, Figs. 13, 15–18), and of *Danae racemosa* (L.), Moench. (1, p. 233, and Fig. 7, A–E, p. 232).

These records, and others which might be cited, show that leaf-traces in which, during part of their course, the xylem encircles the phloem are by no means rare.

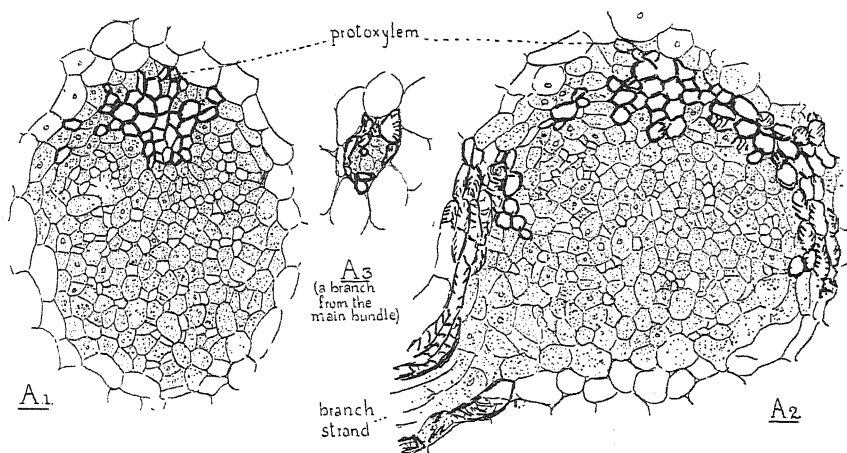
A slightly different type of amphivasal bundle is that met with in the scutellum—the sucking region of the cotyledon—in certain Grasses (19 and 20). It is illustrated for *Zea Mays*, L., and *Coix lacryma-Jobi*, L., in Fig. 11, and I have also seen it in *Hordeum vulgare*, L. In *Zea* the main bundle-trunk of the scutellum, which is collateral at the base (Fig. 11, A₁), tends to become more or less amphivasal in passing up (Fig. 11, A₂). A more completely concentric strand is illustrated in 19, Pl. V, Fig. 18. The scutellum bundle of *C. lacryma-Jobi* is amphivasal, even at the base (Fig. 11, B₁). The main bundle-trunk in the scutellum of both these species gives off branches towards the epithelium, and those who prefer to trace all shoot bundles downwards from above, would describe the amphivasal character as arising through the attachment of successive branches to the dorsal face of the bundle (Fig. 11, A₂ and B₁). But I do not think that this explanation will hold, for there is a strong tendency to amphivasal character, even in the small branch strands, for which such an interpretation can scarcely be valid (Fig. 11, A₃ and B₃).

In the examples hitherto discussed, with the possible exception of the

scutellum bundles, the amphivasal character is not connected with branching (or with what would be called fusions in tracing the bundles downwards). But according to Chrysler (15) the most typical amphivasal bundle is that

AMPHIVASAL BUNDLES OF SCUTELLUM

A, ZEA MAYS, L.



B, COIX LACRYMA-JOBI, L.

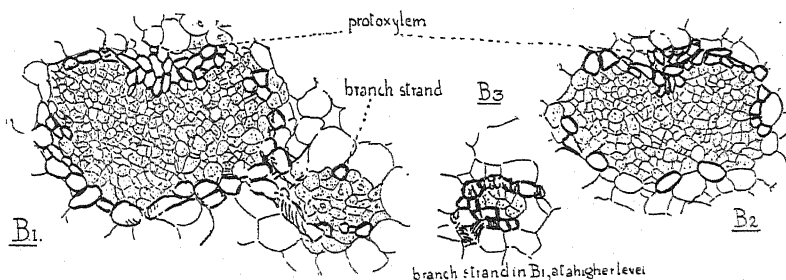


FIG. 11. Amphivasal bundles of the Grass scutellum. Transverse sections from microtome series from below upwards ($\times 193$). A₁–A₃, *Zea Mays*, L. A₁ shows the collateral structure of the base of the bundle; in A₂ an amphivasal structure is approached; an amphivasal branch-bundle is being given off. The phloem and conjunctive tissue of the bundle are dotted, but on the phloem side it is doubtful where the limit of this should be set. A₃ shows an amphivasal branch strand. B₁–B₃, *Coix lacryma-jobi*, L. B₁, showing amphivasal character of main trunk near base; B₂, main trunk, higher; B₃, the branch in B₁ at a higher level.

which originates through fusion of bundles at the nodes. He does not report the exact history of this occurrence in the case of any individual bundles, but merely says that the fact that amphivasal bundles are due to fusion 'has been amply borne out by observation', and he names twelve species of Grasses in which such bundles occur. He says that 'before two bundles of a monocotyledon fuse, they swing around so that phloem fuses with

ACORUS CALAMUS, L.

(Amphivasal bundles for comparison with those of Gramineae)

In series A&B, an amphivasal bundle branches into 2 collateral bundles.

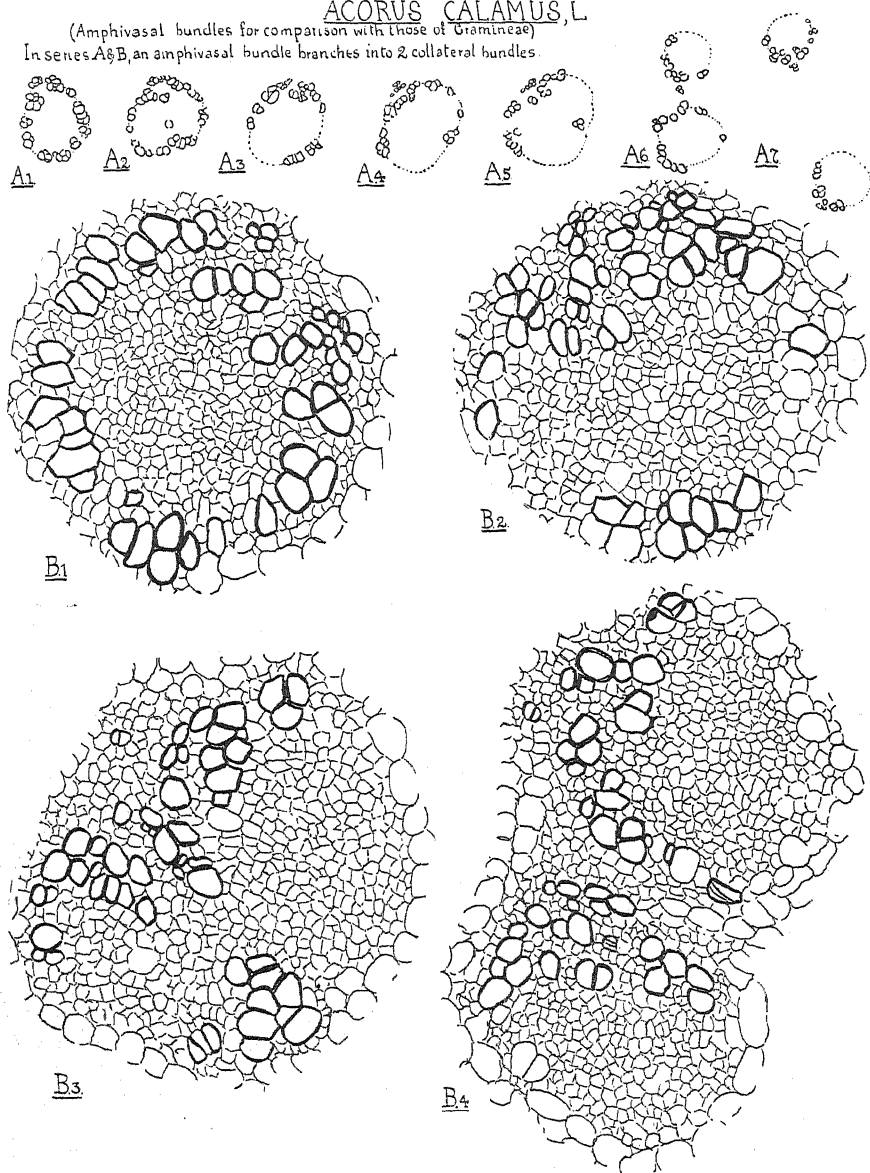


FIG. 12. *Acorus calamus*, L. Transverse sections from a series from below upwards passing through bundles near the apex of a young rhizome. A₁-A₇ ($\times 47$) show the passage from the amphivasal stage (A₁) to the division into two collateral bundles (A₇); semi-diagrammatic, the lignified xylem elements only being indicated. In A₂, there are signs of division which are lost again in passing up. B₁-B₄ ($\times 193$), a series through another bundle, showing the passage from an amphivasal bundle (B₁) to two collateral bundles (B₄).

phloem, and the xylem accordingly surrounds the compound bundle'. Though I have been on the watch for this phenomenon, I have never myself seen it, in the Grasses or elsewhere. As an example of a plant that

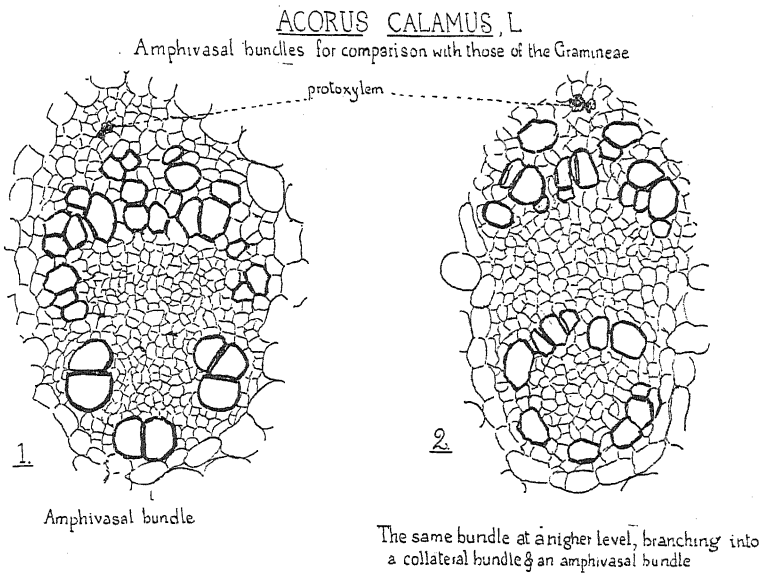


FIG. 13. *Acorus calamus*, L. Two sections from a transverse series passing from below upwards through a rhizome bundle ($\times 193$), to show branching into one collateral and one amphivasal bundle.

might be expected to show it, I examined *Acorus calamus*, L., the Sweet Flag, in which amphivasal bundles are known to divide in passing upwards. I cut two series of microtome sections, one through the apex of a growing rhizome and the other through an older region. In the apex of the rhizome the bundles both in the central cylinder and the cortex are collateral. The first concentric bundle appears a little way from the apex at a level at which the diameter of the rhizome is about 0.85 cms. I followed two of these concentric bundles upwards, and they are illustrated in Fig. 12, A and B. That represented in A_1-A_7 is completely concentric in A_1 , but in A_7 it has branched into two normal collateral bundles. But if its history is followed down from A_7 to A_1 it is seen that the phloems cannot be said to 'swing around' before they fuse with one another, and that the two crescents of xylem do not, by meeting marginally, form a ring, as one would expect from Chrysler's description of concentric monocotyledonous bundles. The same point is illustrated on a larger scale in Fig. 12, B_1-B_4 , for the second bundle whose course was traced.

My other series of sections was through an older part of the rhizome, where the diameter was about 1.3 cms. In this region the great majority of the bundles in the central cylinder are concentric, but a few are collateral,

while those in the cortex are all collateral. Fig. 13, p. 617, shows the process of division of a concentric bundle, traced upwards; it breaks up into one concentric and one collateral strand. I have seen the same process in four other bundles whose history I followed, and the impression I obtained from my sections was that it was the general process. Fusion may be invoked to explain the form of the bundle in Fig. 13, 1, but the neatly concentric character of the lower bundle in Fig. 13, 2, cannot be accounted for in this way.

The comparison of all the amphivasal vascular strands which I have seen in monocotyledonous shoots, and especially in Grasses, leads me to suppose that such bundles are as a rule merely further modifications of single collateral strands—a return to the view expressed long ago by de Bary (11). It does not appear that there is any foundation for describing them as arising *in general* through bundle fusions.

3. SUMMARY.

1. The first part of this paper relates to the origin and history of the plexus of more or less horizontal bundles which characterizes the nodes of Grasses and to the vascular relations of the adventitious roots and lateral buds occurring at the nodes. The species in which the nodal plexus is considered are *Coix lacryma-Jobi*, L. (Fig. 1, p. 595), *Avena sativa*, L. (Fig. 2, p. 597), *Leersia oryzoides*, Sw. (Fig. 3, p. 598), *Phalaris arundinacea*, L. (Fig. 4, p. 599, Fig. 5, p. 601, Fig. 6, p. 603), and *A. barbata*, Brot. (Fig. 7, p. 604, and Fig. 8, p. 605). It is shown that the early connexions between the bud and the nodal plexus are not sufficiently important to justify the view that the nodal plexus originates from the bud supply (pp. 609–12). In its initial stages the supply for the adventitious roots is found to be even less significant in this connexion (pp. 606–7). So the idea that either the bud strands, or the root strands, or both, are responsible for the nodal plexus at its inception, may be dismissed. Bugnon's statement (12) that the horizontal bundles in the node are formed by an abrupt change of direction of the longitudinal leaf-traces must also be discarded *as a generalisation*, though no doubt the course of individual leaf-traces is open to description in this way. The conclusion to which my observations point is that it is a recrudescence of meristematic activity, affecting the ground tissue between the bundles, which results in the formation of the horizontal strands. The origin of the plexus may thus be such that it is open to description as *cauline*. But this activity of the ground tissue may coincide with activity affecting the flanks of the intrafascicular cambium region in the vertical bundles, by means of which connexions are established between the new horizontal strands and the pre-existing vertical leaf-traces (Fig. 5, p. 601, Fig. 8 p. 605). This cell-division in connexion with the leaf-traces may be

so closely contemporaneous with the initiation of the horizontal strands that the plexus may be described as originating through *branching of the leaf-traces*.

Incidentally, attention is drawn to the irregular relation of the vascular supply of the bud to that of the parent axis. The cause of the asymmetry of the vascular supply within the bud in its early stages is also discussed (pp. 607–8). The statement that bud- and leaf-traces always pass through a stage in which they are discontinuous with the vascular skeleton of the parent axis is criticized, and it is held that, as far as the Grasses are concerned, more work is needed on this point (pp. 608–9).

2. In the second part of the paper the amphivasal bundles of Grasses are considered in comparison with those of *Acorus* (pp. 612–18, and Figs. 9, 10, 11), and the conclusion is reached that they represent, as a rule, a special modification of single collateral bundles, and that the statements which have been made as their *general* origin by bundle fusions are not warranted.

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Crossotheca and Lyginopteris oldhamia.

BY

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With Plates XXXIII and XXXIV.

I. INTRODUCTORY.

IN 1903, Professor F. W. Oliver recognized the detached seed known as *Lagenostoma lomaxi* as the fructification of *Lyginodendron* (= *Lyginopteris*) *oldhamia* (21, 22), a discovery which led to the institution of the group Pteridospermeae. The basis of this discovery was the similarity existing between the glands borne on the cupule of the seed and those on the leaves and stems of *L. oldhamia*. Since then a number of Carboniferous fern-like leaves have been found actually bearing seeds. For example, in 1905 Grand'Eury found *Pecopteris pluckeneti* bearing seeds of the *Samaropsis* type, while *Neuropteris obliqua* and *N. heterophylla* have been shown to bear seeds designated *Neuropterocarpus*. In other cases the cupules of seeds have been found attached to leaves, or the constant association between certain seeds and certain leaves strongly suggests that they belonged to each other. On these grounds (sometimes supported by structural or other evidence), it is very probable that in *Trigonocarpus parkinsoni* we have the seed of *Alethopteris lonchitica*, that *Pachytesta gigantea* is the seed of *Alethopteris grandini*, that *Neuropteris gigantea* and species of *Linopteris* bore fructifications referred to *Hexapterospermum*, &c. Although the information is necessarily fragmentary, a large amount is now available with regard to the 'female' organs of the Pteridospermeae.

With regard to the 'male' organs of these plants the situation is much less satisfactory. In 1905 the late Dr. R. Kidston (8, 9, 11) discovered sporangia (referable to the genus *Crossotheca*, Zeiller) in organic connexion with impressions of leaves which he identified as *Sphenopteris hoeninghausi*, Brongniart, which is again identical with the (structurally preserved) leaves of *Lyginopteris oldhamia*.¹ The specimens on which Kidston's discovery

¹ There can be no doubt as to the identification of the petrified leaves of *L. oldhamia* with impressions of leaves designated *Sphenopteris hoeninghausi*. Their identity was first suggested by Professor Williamson, while Kidston (op. cit., 1906) adduced evidence based on the shape of the

was based were collected by Mr. H. W. Hughes from the Ten-foot Ironstone Measures (Yorkian Series = the 'Westphalian' of Kidston = Middle Coal Measures) at Coseley, near Dudley, South Staffordshire. Until this time *Crossothea* had been referred to the Marattiaceae,¹ but Kidston's work received general acceptance, and *Lyginopteris oldhamia* came to be regarded as one of the best known of fossil plants (20, 24, 27, 28). It should be added that we have little satisfactory information with regard to the microsporangia of the other known members of the Pteridospermeae, and that this, the first discovered, case therefore remains the most important.

It is a matter for some concern, therefore, that two palaeobotanists have expressed disagreement with Kidston's conclusion that in *Crossothea* we have the microsporangia of *Lyginopteris oldhamia*. Mr. W. Hemingway, of Derby, differed from Kidston on this point from the first, although he did not publish the evidence on which his view was based. Professor W. Gothan first expressed disagreement in 1913 (5), and he has recently (6) amplified his statements. We have therefore to thank Professor Gothan for calling attention to a question of the first importance. Before restating the grounds of his disagreement it may be added that, in 1908, Professor R. Chodat (4) suggested that the annulate sporangia occurring in 'coal-balls', and referred by Dr. Scott to *Pteridotheca*, belonged to *Lyginopteris*. As Dr. Scott (23, 24) has pointed out, however, the leaflets on which *Pteridotheca* were borne are quite distinct from those of *Lyginopteris*, so this suggestion can be dismissed at once.

Professor Gothan makes the following statements:

(1) That Kidston's identification of the sterile leaves of the Dudley specimens with Brongniart's *Sphenopteris hoeninghausi* was in error; he says that no indubitable example of *S. hoeninghausi* has been found associated with the *Crossothea* from Dudley.

(2) That the horizon of the Dudley *Crossotheas* (i. e., Yorkian Series) is too high for it to represent the microsporangia of *Lyginopteris oldhamia* (which, as a structural species, is found in the 'coal-balls' from the Lanarkian Series only).

(3) That the plants with which the Dudley *Crossothea* is associated are different from those with which *Sphenopteris hoeninghausi* is associated.

Mr. Hemingway's objections, for which I am indebted to him in a series of letters, are somewhat different, and are best dealt with separately (Sections V and VI, below).

leaves, their mode of attachment, their venation, the occurrence of spines on both foliage and stems, and the reticulate outer cortex of the latter. He pointed out that *S. hoeninghausi* and *L. oldhamia* are the only known plants from the same horizon each possessing all these characters. I do not know of any instance of this identification being called in question.

¹ On account of the exannulate nature of the sporangia and the fact that they are slightly united.

At Dr. Scott's suggestion, I have re-examined the available evidence. For the sake of clearness, and indeed of accuracy, it will be necessary to rename the *Crossotheas* which Kidston called *Crossothea hoeninghausi*, and referred to *Lyginopteris oldhamia*. Mr. Hemingway has suggested the name *Crossothea kidstoni*, and this name is here adopted.

In the course of this investigation I have very carefully examined all specimens of *C. kidstoni* and of *S. hoeninghausi*, Brongniart, in the following collections:

(1) The Kidston Collection (Museum of Practical Geology). This includes examples of *C. kidstoni* from Dudley and of *S. hoeninghausi* from various English and Scottish localities, as well as from Belgium and U.S.A.

(2) The collection of Mr. H. W. Hughes, F.G.S., of Dudley, containing the original specimens (and others) on which Kidston based his determination.

(3) The collection of the British Museum (Natural History), including some examples of *Crossothea* from Dudley and of *S. hoeninghausi* from Westphalia, Silesia, Moravia, and U.S.A.

II. THE IDENTIFICATION OF *C. KIDSTONI* WITH *S. HOENINGHAUSI*.

I understand from Mr. Hughes, the collector of the critical specimens, that Kidston's reference of the sterile leaves (which were found both attached to, and associated with, the *C. kidstoni*) to Brongniart's *S. hoeninghausi* was based on some hundreds of specimens which he saw over a long period of years before announcing his discovery. Only the best examples were kept, and the majority of these, including all the figured specimens, are in Mr. Hughes's private collection. The late Professor Zeiller, who, apart from Kidston, had no equal in dealing with Carboniferous impressions, accepted the identification after some preliminary doubt.¹ Professor A. C. Seward (29), after examining Kidston's specimens, also agreed. He concluded that, 'although the available data appear to favour the view generally held, that Kidston's conclusion is correct, additional evidence would be welcome'.

In re-examining the specimens, and comparing them with foreign examples and various plates of *S. hoeninghausi*, Brongniart, my experience was that of Zeiller; at first I was inclined to doubt, but the more specimens I examined the more I became convinced that the Dudley sterile leaves are indistinguishable from Brongniart's species.

S. hoeninghausi is a very variable species. This is due to several factors, the first two of which were pointed out by Kidston:

(1) Normally the pinnules are convex, but in the case of specimens preserved in shales they are often flattened by pressure. It follows that

¹ I am indebted to Dr. Scott for this information.

uncompressed examples appear to bear more rounded segments than those which have been flattened (where the apex often appears truncate).

(2) Irrespective of the presence or absence of pressure, the pinnules fall into two main types, one bearing more or less rounded lobes (as figured by Brongniart (3) (see Pl. XXXIII, Fig. 1), and the other being more cuneate, as figured by Andrae (1), (see Pl. XXXIII, Fig. 2).

(3) A further possible source of apparent variation in the leaflets, not hitherto noted, consists in the fact that all the Dudley leaves (*C. kidstoni*) are preserved in ironstone nodules, and most or all the available examples of Brongniart's *S. hoeninghausi* with which they have to be compared are preserved in shales. A shale containing a convex leaf will split readily, and give a good and little-distorted impression (apart from pressure, noted above). On the other hand, an ironstone nodule splits irregularly, with much less reference to the line of weakness caused by the included fossil; as a result, especially in the case of a convex leaf, part of the lamina may come away on one half of the nodule and part on the other, giving a distorted impression on both halves. In some instances, therefore, there is a tendency for only the lamina which is adjacent to the veins to be shown on one (or even on both) halves of the nodule. This partly accounts for the appearance of some of the more linear segments in the Dudley specimens. The effect is seen, for example, on specimen No. XV. 1373, British Museum (Natural History), the two halves of which are figured on Pl. XXXIV, Figs. 11 and 12. (Compare the pinnules indicated by arrows.) Better examples than these can be produced, but it is seldom that a satisfactory photograph can be obtained of both halves of the nodule, even where these have been preserved. The specimens figured on Pl. XXXIV at Figs. 14 and 18 have clearly suffered from this cause. At all events, it will be evident that it is essential in all possible cases to examine both halves of a nodule.

While in the Dudley nodules the cuneate type of foliage is commoner than that with rounded lobes, there are all transitions between the two forms, and this is especially significant as occurring in specimens derived from a single locality and horizon. The extreme cuneate type is shown on Pl. XXXIII, Figs. 5 and 6; here, indeed, the pinnules are almost linear, though the effect is no doubt due in part to the splitting of the nodule. In the relatively narrow pinnule segments these specimens approach *Crossothea schatzlarensis* (Stur), (14), a species which is rare in Britain except in the Yorkshire Coalfield, and is unknown from any horizon in the South Staffordshire Coalfield.¹ The cuneate type of leaf is figured on Pl. XXXIII,

¹ Mr. Hemingway is of the opinion that the barren leaves of *Crossothea kidstoni* merge into those of *C. schatzlarensis*, occurring in ironstone nodules and shales in Yorkshire. He observes that the leaves of the latter species can be traced from the ironstone nodules in which they occur into the surrounding shale, and that the plants are one-third larger in the former than in the latter (the difference being due to compression). I cannot accept this view, although I have not seen the

Fig. 7 (cf. Andrae's Fig.), while in the specimen figured on Pl. XXXIII, Fig. 4, the lobes are slightly broader. This forms a transition between the cuneate form and that with rounded lobes, figured on Pl. XXXIII, Fig. 3 (which should be compared with Brongniart's figure of his species—Pl. XXXIII, Fig. 1). I should have no hesitation in identifying this specimen with *Sphenopteris hoeninghausi*, Brongniart. Pl. XXXIII, Fig. 10, shows an example in which the lower pinnules tend to bear rounded lobes, while the upper pinnules are of a more cuneate type. *I can thus find no character by which the sterile leaves of C. kidstoni can be separated from Brongniart's S. hoeninghausi.* The Dudley nodules contain examples of both forms of that species, together with all transitions between them.¹ I attempted to obtain cuticle preparations from the Dudley plants, with a view to comparing them with similar preparations from Lanarkian examples of *S. hoeninghausi*, but without satisfactory results.

III. THE HORIZON OF *C. KIDSTONI*.

Apart from the Dudley leaves, which are under review, specimens which must be identified with Brongniart's *S. hoeninghausi* occur at several localities in the Yorkian rocks of Britain. One of the best of these is from the Three-quarter Coal of Chopwell, near Ebchester, Co. Durham (figured on Pl. XXXIV, Figs. 13, 20). It is almost identical with the specimen figured as *S. hoeninghausi* by Stur (30). Other good specimens of Yorkian age are preserved in the Kidston Collection—Nos. 939 and 940 (Pl. XXXIV, Figs. 8 and 9 and 19 respectively). These are from Netherton, South Staffordshire. Again, a specimen in the Kidston Collection (No. 5173) from Chopwell (not here figured) agrees well with Brongniart's drawing. It may here be pointed out that the Yorkshire specimens recorded by Kidston (17, 31) as of Yorkian age may prove to have been of Lanarkian age.² It is clear, how-

specimens to which Mr. Hemingway refers. No plant has been identified from Dudley as *C. schatzlarensis*, and the segments of that species are more filamentous than the most linear segments found of *C. kidstoni*. Kidston (op. cit., 1923-5, p. 341) observed: 'In the sterile condition *Crossothea schatzlarensis* could not be easily mistaken with any other Upper Carboniferous species, the long, narrow filamentous segments of the pinnules being very distinctive.' The fruiting pinnules, however, have a great similarity to those of *Crossothea hoeninghausi*, Brongt., sp. (in the present paper called *C. kidstoni*) but are smaller. It may be added that Mr. Hemingway regards the rachis of *C. kidstoni* as being longitudinally striated, as in *Neuropteris* (a point which will be dealt with later), while that of *C. schatzlarensis*, according to him, is smooth and shiny.

¹ It should be noted that Zeiller's enlargement of a leaflet of *S. hoeninghausi*, showing the form of the leaf and its venation (Zeiller, R., 'Flore Fossile du Bassin Houiller de Valenciennes', Études des Gîtes Minéraux de la France, Paris, 1890, Pl. VI, Fig. 2 a) differs from that of Brongniart (loc. cit.) in the venation: in Zeiller's figure the veins are shown as being straight and almost stick-like, but in Brongniart's they are curved, as would be expected in a convex leaf. Yet the margins of Zeiller's leaflet are shaded, indicating the convexity of the lamina. The Dudley specimens have curved veins, as figured by Brongniart.

² Mr. Hemingway would refer these to the Lanarkian Series.

ever, that foliage indistinguishable from Brongniart's *S. hoeninghausi* did occur in British rocks of Yorkian (as well as of Lanarkian) age.

IV. THE PLANTS ASSOCIATED WITH *C. KIDSTONI*.

The following plants have been recorded by Kidston (13) from the same horizon as the specimens of *C. kidstoni*, i.e., the Ten-foot Ironstone Measures (roof of Thick Coal—Yorkian Series) of Coseley, near Dudley. Those species which are also known from the Lower Coal Measures (Lanarkian Series) of other British Coalfields are marked by a x :

FERNS AND PTERIDOSPERMS.

<i>Asterotheca miltoni</i> , (Artis)	x
<i>Dactylothea plumosa</i> , (Artis)	x
<i>Mariopteris nervosa</i> , (Brongt.)	x
<i>Alethopteris lonchitica</i> , (Schloth.)	x
" <i>decurrens</i> , (Artis)	x
" <i>davreuxi</i> , (Brongt.)	x
" <i>valida</i> , (Boulay)	x
" <i>serli</i> , (Brongt.)	x
<i>Lonchopteris rugosa</i> , (Brongt.)	x
<i>Neuropteris heterophylla</i> , Brongt.	x
" <i>gigantea</i> , Sternb.	x
" <i>obliqua</i> , (Brongt.)	x
" <i>osmundae</i> , (Artis)	x
" <i>carpentieri</i> , Kidston	x
<i>Aphlebia crispa</i> , (Gutbier)	? x

EQUISETALES.

<i>Equisetites hemingwayi</i> , (Kidston)
<i>Calamites schütziformis</i> , K. and J. forma <i>waldenburgensis</i> , Kidston
pro sp.	x
" <i>carinatus</i> , Sternb.	x
<i>Asterophyllites equisetiformis</i> , (Schloth.)	x
" <i>longifolius</i> , (Sternb.)	x
" <i>grandis</i> , (Sternb.)	x
" <i>charaeiformis</i> , (Sternb.)	x
<i>Annularia radiata</i> , Brongt.	x
" <i>galioides</i> , (L. and H.)	x
" <i>sphenophylloides</i> (Zenker)	x

SPHENOPHYLLALES.

<i>Sphenophyllum cuneifolium</i> , (Sternb.)	x
" <i>tenuissimum</i> , Kidston	x

LYCOPODIALES.

<i>Lepidodendron ophiurus</i> , Brongt.	x
<i>Lepidostrobis variabilis</i> , L. and H.	x
<i>Bothrodendron minutifolium</i> , (Boulay)	x
<i>Sigillaria tessellata</i> , Brongt.	x
<i>Lepidocarpon westphalicum</i> , Kidston	x

CORDAITALES.

<i>Cordaites borassifolius</i> , (Sternb.)	x
" <i>principalis</i> , (Germar.)	x
<i>Artisia approximata</i> , (Brongt.)	x
<i>Cordaianthus volkmanni</i> , (Ett.)	x

SEEDS, &c.

<i>Polypterocarpus anglicus</i> , (Kidston.)	
„ <i>johnsoni</i> , (Kidston.)	
„ <i>ornatus</i> , (Arber.)	
<i>Lagenospermum oblongum</i> , (Kidston.)	
„ <i>urceolaris</i> , (Kidston.)	
<i>Holcospermum elongatum</i> , (Kidston) x
<i>Rhabdocarpus renaulti</i> , Kidston.	
„ <i>oliveri</i> , Kidston.	
<i>Megalospermum wildi</i> , (Kidston.)	
<i>Trigonocarpus noeggerathi</i> , (Sternb.) x
<i>Hexagonocarpus hookeri</i> , Kidston.	
<i>Whittleseya elegans</i> , Newberry.	
„ <i>fertilis</i> , Kidston.	

Professor Gothan claims that the plants with which *C. kidstoni* is associated are different from those with which *S. hoeninghausi* is associated, the latter being earlier forms.

If the identity of the plants mentioned in Section III (i. e., Kidston Collection, Nos. 5174, 939, 940, and 5173) with *S. hoeninghausi*, Brongt., is admitted, this argument falls to the ground. In this connexion I may observe that Mr. Hemingway has (*in litt.*) acknowledged that Kidston's (Pl. LXXXVII, Fig. 4, i. e. specimen No. 5174) represents a typical example of Brongniart's species.

It will be seen, however, from the above lists, that the majority of the plants associated with *C. kidstoni* are also known from the Lanarkian Series. Seeds and sporangia are usually so rare and restricted in lateral distribution as to be of little zonal value. Thus, *Polypterocarpus anglicus*, *P. johnsoni*, *Lagenospermum oblongum*, and *L. urceolaris*, *Rhabdocarpus oliveri*, *Whittleseya fertilis* and *W. elegans* are recorded in Britain from this horizon and locality only, and in some cases but one specimen is known. *P. ornatus*, *R. renaulti*, *Megalospermum wildi*, and *Hexagonocarpus hookeri* are all rare, only the first named having been found outside the Yorkian Series. *Neuropteris carpentieri* and *Sphenophyllum tenuissimum* are represented in Britain by the single record from Dudley.

Lonchopteris rugosa, on the other hand, has not yet been found in the Lanarkian, while *Alethopteris davreuxi*, *A. serli*, and *Annularia sphenophylloides* are very rare in the Yorkian and unknown from the Lanarkian. Kidston (op. cit., 1914, pp. 102, 103, 122) records these from Dudley as 'not common', 'very rare', and 'very rare' respectively, so that their occurrence may be regarded as more or less accidental—at least they do not constitute a prominent feature of the flora. *The plants associated with C. kidstoni do not assist in deciding for or against Kidston's determination.*

V. THE STRIATIONS ON THE RACHIS OF *C. KIDSTONI*.

Mr. Hemingway suggests (*in litt.*) that the rachis of *C. kidstoni* is distinct from that of *Lyginopteris oldhamia* in that the striations are

longitudinal in the former (as in the Neuropterideae), while, as is well known, in the latter they form a meshwork. I have very carefully examined all available rachises of *C. kidstoni*, and while in many cases the striae are obscure, this statement does not accord with my observations. In several instances the striae on specimens in Mr. Hughes's collection do not take a straight course, and they must have united (though in these cases actual union cannot be observed). But in the specimen figured on Pl. XXXIV, Fig. 10, the rachis is seen to be reticulate. (In small stems, such as these, the meshes are necessarily small, and can be seen only with the aid of a lens). It should also be pointed out that the closely similar *C. hughesiana* (which differs from *C. kidstoni* only in the size and shape of the fertile pinnules—the sterile pinnules being as yet unknown) had a reticulate rachis. For example, the specimen of *C. hughesiana*, part of which was figured by Kidston (19), distinctly shows this feature on the main rachis (though the part there reproduced does not include the rachis). This specimen is in Mr. Hughes's collection. As he regarded the rachis of *C. kidstoni* as being longitudinally striated, Mr. Hemingway would refer it to the Neuropterideae, but the specimen figured rules out the possibility. *There is no evidence in the striations on the rachis to separate C. kidstoni from Lyginopteris.*

VI. THE FORM OF THE STERILE LEAVES OF *C. KIDSTONI*.

Kidston (10), in 1906, gave a table of eight species of *Crossothea*, showing that in four instances (which included *C. kidstoni*) the sterile foliage was Sphenopteroid, in three they were Pecopteroid, while in *C. hughesiana* they were unknown. Mr. Hemingway, however, observes (*in litt.*): 'The *Crossotheas*, in all cases where proof could be obtained, have been found to be Pecopteroid and not Sphenopteroid plants. This, I believe, will be found to hold good for the whole group.' If this is true, *C. kidstoni* could not belong to *L. oldhamia*, the sterile foliage of which, when found as impressions, is called *S. hoeninghausi*. The main interest, therefore, centres round *C. kidstoni*, and Mr. Hemingway considers that the barren leaves of this species are reduced Pecopteroid leaves. To me they appear to be typically Sphenopteroid, both in shape and venation. (See, for example, Pl. XXXIII, Fig. 3, Pl. XXXIV, Fig. 10.)

Turning to the other known species of *Crossothea*, *C. schatzlarensis* (Stur) is clearly of the Sphenopteroid (Rhodean) type. *C. communis* (Lesqx.) (15) Mr. Hemingway regards as 'certainly a Pecopteris'. He has examined specimens, both in his own collection and in that of Mr. W. R. Barker of Barnsley, and observes: 'None of our specimens agree with Kidston's restoration of the barren foliage. Extreme forms have a somewhat Sphenopteroid appearance, and this is considerably exaggerated in Kidston's drawing. From the Sphenopteroid form there is a gradual

transition to the normal form of the pinnules in *Asterotheca miltoni*.¹ In the fruiting pinnae there is also a gradual transition from *A. miltoni* (with the sporangial groups attached to the backs of the leaves) to *C. communis*

Species of Crossotheca.

Species.	Distribution.	Type of Sterile Leaves.
<i>C. pinnatifida</i> , (Gutbier)	Radstockian and Staffordian Series	Pecopteroid.
<i>C. sagittata</i> , (Lesqx.)	Radstockian Series	Pecopteroid (allied to <i>P. miltoni</i> (Artis)).
<i>C. ophioglossoides</i> , (Lesqx.)	U.S.A.	Pecopteroid.
<i>C. boulayi</i> , Zeiller	Staffordian and Yorkian Series.	Pecopteroid.
<i>C. trisecta</i> , Sel- lards	U.S.A.	Pecopteroid.
<i>C. communis</i> , (Lesqx.)	Yorkian Series	?Pecopteroid.
<i>C. schatzlarenensis</i> , (Stur.)	Yorkian Series	Sphenopteroid (rhodean type).
<i>C. kidstoni</i>	Yorkian Series	Sphenopteroid, with cuneate or rounded lobes.
<i>C. hughesiana</i> , Kidston	Yorkian Series	Unknown.
<i>C. crépini</i> , Zeiller	Staffordian and Yorkian Series.	Sphenopteroid, with rounded lobes.

(with the leaf-tissue completely aborted and the sporangia pendent at the ends of the naked veins).’ Mr. Hemingway kindly supplies drawings of certain specimens of this species, and adds: ‘The foliage of *C. communis* is densely hirsute (identical with that of *A. miltoni*). When freshly exposed on the stone, the hairs can often be distinctly seen. Kidston mentions the peculiar striated appearance of the surface of the leaves, but in his *Memoir* he expresses some doubt as to this being due to hairs. The specimens in the Kidston Collection are extreme forms, and will not give the evidence needed. The intermediate connecting links are necessary.’ He is endeavouring to obtain further examples of *C. communis* with a view to establishing this point. It will be seen that Mr. Hemingway regards *C. communis* as only a condition of preservation of *A. miltoni* (he adds, Artis,

¹ It should be noted that Kidston (10) describes the barren leaves of *C. sagittata* (Lesqx.) as being allied to those of *Asterotheca miltoni*.

not Kidston). I have not seen the specimens on which these observations have been made: Kidston's drawings show the leaves as Sphenopteroid.

With regard to *C. boulayi*, Zeiller, the pinnules are typically Pecopteroid. Kidston (10) originally described them as 'Sphenopteroid with rounded lobes', but later (16) called them Pecopteroid. *C. crépini*, Zeiller, is considered by Mr. Hemingway as being more Pecopteroid than Sphenopteroid. Kidston described it as the latter, and with this I agree. The various species of *Crossothea*, together with the type of sterile leaf borne, are given in the table on p. 629. There can be little doubt that at least two main groups of plants are represented.

The form of the sterile leaves of C. kidstoni was Sphenopteroid, and does not militate against the reference of that fructification to Lyginopteris oldhamia.

VII. NEGATIVE DESTRUCTIVE EVIDENCE AND CONCLUSIONS.

Although the barren leaves known as *S. hoeninghausi* (admittedly identical with the leaves borne by *L. oldhamia*) may be fairly common on certain Coal Measure horizons, both in Britain and elsewhere,¹ fertile fronds of *C. kidstoni* are very rare, or entirely absent, except at Dudley. Indeed the only fertile specimen of *C. kidstoni* known to me, apart from those in the Dudley nodules, is that collected by the Rev. R. H. Goode from the Yorkian Series of Lea Green Colliery, Thatto Heath Station, Lancashire (Kidston Collection, No. 6317), and we thus have no Lanarkian records of fertile examples of *C. kidstoni*.

Again, although the 'coal-balls' of Lancashire and Yorkshire (occurring in the Lanarkian Series—Lower Coal Measures) are rich in *L. oldhamia* (and contain examples of *Lagenostoma lomaxi*, which has, no doubt correctly, been referred to that species) *not one example of a true Crossothea has been recorded from these 'coal-balls'* (25). It is true that Professor Seward (28) figured a section from the Coal Measures of Oldham which 'is probably a bilocular sporangium of the same type as those described by Kidston from Dudley', while Dr. Scott (25) provisionally referred a petrified bisporangiate synangium, the parent plant of which is as yet unknown, to the genus *Telangium*. *The absence of a true Crossothea from Lyginopteris-bearing 'coal-balls' precludes the reference of C. kidstoni to L. oldhamia. This is the only valid evidence against Kidston's conclusion, but it is in itself fatal to that conclusion.*

¹ With regard to the distribution of *S. hoeninghausi*, Kidston (op. cit., 1923-5, p. 334) observes that it 'occurs both in the Westphalian Series and the Lanarkian Series. It is very irregularly distributed, and from several of the coalfields has not been recorded. In some where it has been met with it is rare, while in the "coal-balls" of Lancashire and Yorkshire it is very common, and at Tullygarth Pit, near Clackmannan, it was so abundant in a band of blaes passed through when sinking the shaft that it was suggestive of the plants having grown in a dense mass or thicket'.

The fructification standing nearest to *C. kidstoni* which occurs in 'coal-balls' is *Telangium scotti*, Benson (2), a not uncommon species. It is of about the same size and shape as *C. kidstoni*, with which it may be compared as follows :

<i>C. kidstoni</i> .	<i>T. scotti</i> .
Bilocular.	Unilocular.
More free at base.	United at base.
Sporangia pendent from underside of an oval disc (representing the pinnule).	Sporangia upright on a disc (? representing the rachis).
Sporangia project beyond the margin of the disc.	Sporangia do not project beyond the margin of the disc.

Dr. Scott (25) did not regard *Crossothea* (which occurs as casts and impressions only) as being very different from *Telangium*, and it is very probable that they belong to the same genus. He observes : ' Dr. Benson described the synangia as borne terminally on the ultimate ramifications of a rachis, without a limb, this being a distinctive character of her genus *Telangium*. As a matter of fact, the synangia of *T. scotti* are often seated on a flat disc or lamina, which may be compared to a fertile pinnule of *Crossothea*, so that the distinction between the genera is not always as marked as it appears at first sight.'

Although Dr. Benson thought *T. scotti* belonged to *Lyginopteris* no case has yet been made out of organic connexion between the two. Kidston, basing his opinion on the structure¹ of *T. scotti*, would not accept it as the male organ of *L. oldhamia*, though he regarded it as belonging to a Pteridosperm.²

Dr. Scott disposed of the claim that *Telangium* differed from *Crossothea* in the presence or absence of a limb. The projection or non-projection of the ripe sporangia beyond the margin of the disc, noted by Kidston (18), can have no generic value, nor can slight differences of union. There

¹ The objections advanced are (1) the microsporangia of *T. scotti* are not attached to a limb, and (2) they are uni- and not bilocular. These are dealt with in the text.

² It was mainly the similarity between the fructifications borne by the *Cyatheetes-Pecopterids* (and especially of *Acitheca* and *Scoleopteris*) and *Telangium scotti* which led Kidston to regard that group as Pteridosperms. Until 1923 they had generally been accepted as ferns with affinities to the Marattiaceae, a view which will probably be upheld by subsequent research. Dr. Scott has reviewed the present situation with regard to this important problem. (See *Nature* for March 2 and 9, 1929, and Notes on Palaeozoic Botany, 1907-29, Recueil des Travaux botaniques néerlandais, vol. xxv a, 1928, p. 365.)

Mr. Hemingway, while regarding *Telangium* as a close relative of *Crossothea*, would not refer it to *Lyginopteris*, his objection (as in the case of *C. kidstoni*, noted above) being based on his observations of the stalks. He says that the stalks of *Telangium* bear longitudinal striations, as in the Neuropterideae.

remains, therefore, only the fact that *C. kidstoni* (and *C. hughesiana*) have been found to be bilocular, and this interpretation of the structure must be admitted, while *T. scotti* is unilocular. The condition in the remaining species of *Crossotheca* is as yet unknown. As pointed out above, however, petrified bilocular sporangia and bisporangiate synangia are known from the Lancashire 'coal-balls'. *C. kidstoni* and *T. scotti* in all probability belong to the same genus.

Professor Gothan regards the *Crossothecas* generally as ferns: Mr. Hemingway suggests that they are the fructifications of Pteridosperms, though not of *Lyginopteris*. The writer considers the resemblance of the sterile leaves of *C. kidstoni* to *S. hoeninghausi* (the foliage of *L. oldhamia*) to be so great that, though *C. kidstoni* cannot have belonged to *L. oldhamia*, it must have belonged to another species of *Lyginopteris*. *S. hoeninghausi*, Brongniart, as at present understood,¹ represents the foliage of at least three distinct, though related, species. It is possible, on the grounds of association, to suggest the corresponding fructifications in one or two cases, though at present this can be little more than suggestion. The seed *Lagenospermum oblongum* (Kidston) occurs in association with *C. kidstoni* in the Ten-foot Ironstone Measures of Dudley (12), and is the only fructification known from this horizon and locality which can be definitely referred to the genus *Lagenospermum*. (This genus includes seeds occurring as casts and impressions, possessing a cupule, and having a general resemblance to *Lagenostoma*, but showing no structural features.) It is therefore probable that *L. oblongum* was the seed of *C. kidstoni*. The identity of the glands on *L. lomaxi* with those on the leaves, &c., of *L. oldhamia*, as shown by Professor Oliver, leaves no doubt that we have here the seed of that species, while Dr. Benson's *T. scotti* may well have represented the microsporangia of that plant, as she suggested. In support of this are several features noted by that author: (i) *Telangium* occurs in 'coal-balls' associated with *L. oldhamia*²; (ii) there are certain structural similarities in the two; (iii) the spores found in the pollen chamber of *L. ovoides*, Williamson, greatly resemble those of *Telangium*; (iv) the apex of *Lagenostoma*, as seen in transverse section, has some resemblance to *Telangium*. We therefore have at least three species of *Lyginopteris*, each bearing

¹ I fail to see how the various forms included under that name can be satisfactorily separated.

² The known distribution of *Telangium* and *Lyginopteris*, both as regards the localities from which they have been obtained and their relative frequency at those localities, is significant. I have to thank Mr. J. Lomax for these. He bases the frequencies by counting *Lyginopteris* at Shore as '100'. They are as follows:

	Shore.	Bacup.	Burnley.	Oldham.	Huddersfield.
<i>Lyginopteris</i>	100	60	50	50	10
<i>Telangium</i>	30	15	—	5	—

The following are the distributions of the critical species found in 'coal-balls' on the continent

foliage of the *S. hoeninghausi* type, and the writer suggests that the corresponding male and female fructifications are as given below :

Species.	Microsporangia.	Seeds.
<i>Lyginopteris oldhamia</i> , Binney. A structural species— Lanarkian.	<i>Telangium scotti</i> , Benson.	<i>Lagenostoma lomaxi</i> , Oliver and Scott.
<i>Lyginopteris</i> , sp. <i>A</i> . A structural species— Lanarkian.	Unrecognized.	<i>Lagenostoma ovooides</i> , Williamson.
<i>Lyginopteris</i> , sp. <i>B</i> . Incrustation species— Yorkian.	<i>Crossothea kidstoni</i> .	<i>Lagenospermum oblongum</i> , Kidston.

I wish to thank Dr. Scott, who called my attention to this problem, and who has given valuable advice and encouragement in the work. The position, as it now stands, is not, of course, entirely satisfactory, but further evidence is necessary before more definite statements can be made.

Since writing the above I have been able to examine the slides in the Department of Botany, University College, Gower Street, and to discuss them with Professor E. J. Salisbury. It appears that, unless the position of the sporangia can be definitely made out, it is difficult at present to distinguish between *Crossothea* and *Telangium* in petrified material. There are, however, two distinct types of *Crossothea*-*Telangium* fructification : (*a*) a not uncommon type consisting of a number of sporangia with thin walls and clearly representing *Telangium*, and (*b*) a rare form with thick walls and few sporangia, the two known examples being that figured by Dr. Scott (*Studies*, vol. ii, p. 79) and an unfigured specimen. Dr. Scott's specimen

as given by Dr. R. C. Koopmans in W. J. Jongmans' 'Flora en Fauna van het Nederlandsche Karboon' I: Researches on the Flora of the 'coal-balls' from the 'Finefrau-Nebenbank' Horizon in the Province of Limburg (The Netherlands); Geologisch Bureau voor het Nederlandsche Mijngebied, 1928, p. 2. The horizons and localities, here numbered, are as follows: 1 = Domaniale Mijn; 2 = Rheinpreussen; 3 = Bouxharmont; 4 = Jupille; 5 = Finefrau-Nebenbank; 6 = Vollmond; 7 = Maria; 8 = Katharina.

Dr. Hirmer (7) does not record *Lagenostoma* or *Telangium* from the continent.

	1	2	3	4	5	6	7	8
<i>Lagenostoma lomaxi</i>	—	—	—	—	—	—	—	—
<i>Lagenostoma ovooides</i>	x	x	—	—	x	—	x	x
<i>Telangium scotti</i>	x	—	—	—	x	—	—	—
<i>Crossothea</i>	—	—	—	—	—	—	—	—
<i>Lyginopteris oldhamia</i>	x	x	x	x	x	x	x	x

It will be seen that, as in Britain, there are as yet no continental records from 'coal-balls' of *Crossothea*, and that the seed *Lagenostoma lomaxi* is unknown (it is extremely rare in Britain, and has been found at two localities only—Shore and Bacup), while *L. ovooides* (known from Shore, Bacup, Burnley, Oldham, and Huddersfield in Britain) is recorded from several continental localities. Special interest attaches to *Telangium scotti*, which is seen to accompany *Lyginopteris oldhamia* at two of the continental localities.

was a synangium consisting of two sporangia, each of which was divided by a partition into two loculi. He provisionally placed it in *Telangium*. The second specimen with thick walls was probably unilocular (though, owing to the oblique plane of the section, a bilocular construction could not be definitely ruled out), and this should probably also be referred to *Telangium*. A portion of the leaf-blade was attached below the sporangium and the position of the chlorenchyma suggested that the sporangia were pendent from a definite disc. It was in close association with a small rachis of *Lyginopteris oldhamia* bearing a typical glandular hair. The only record of a fructification from 'coal-balls' with definite affinities to *Crossotheca* is therefore already noted as figured by Professor Seward.

A comparison of the spores showed that there was no marked difference in size or surface ornamentation between those in the second robust type of sporangium (here provisionally referred to *Telangium*) and those in the true thin-walled *Telangium*, while these again resembled those in the pollen-chamber of the seed *Lagenostoma lomaxi*.

VIII. SUMMARY.

1. Kidston's reference of *Crossotheca* to *L. oldhamia* is not vitiated by (a) misidentification of the sterile leaves, (b) the horizon of the specimens, (c) the associated plant remains, (d) the markings on the rachis, (e) the character of the sterile fronds.

2. Nevertheless, the (apparent) total absence of true *Crossotheca* from 'coal-balls' rich in *L. oldhamia* precludes its being the microsporangia of that species.

3. Kidston's *Crossotheca hoeninghausi* is therefore renamed *C. kidstoni*.

4. The great similarity of the sterile leaves borne by *C. kidstoni* to those borne by *L. oldhamia* (i. e., to *Sphenopteris hoeninghausi*) indicates that, though *C. kidstoni* cannot have belonged to *L. oldhamia*, it did belong to another, closely related, species of *Lyginopteris*.

5. It is suggested that in Dr. Benson's *Telangium scotti* we have the microsporangia of *L. oldhamia*, while a second Lanarkian species, also based on structural features, bore the seed *Lagenostoma ovoides* (microsporangia as yet unrecognized). Finally, the plant which bore *Crossotheca kidstoni* (known as incrustations from the Yorkian Series) is regarded as a species of *Lyginopteris*, the corresponding seed probably being represented by *Lagenospermum oblongum*.

ADDENDUM.

While this paper was in proof, I had a visit from Dr. W. J. Jongmans. He informed me that, on the basis of numerous specimens in his possession,

he considers that the sterile leaves of Kidston's *Crossothea hoeninghausi* (called above *C. kidstoni*) is identical with *C. schatzlarensis*, Stur, and most probably also with the European specimens named by Kidston *C. communis* (which may or may not be identical with the plant figured by Lesquereux under that name). Should this prove to be true, we could unite under *C. kidstoni* both *C. schatzlarensis* and *C. communis*, retaining the latter two names for extreme types only of the more comprehensive species.

The specific name '*schatzlarensis*' is most unfortunate: there are at least four Sphenopteroid fronds bearing that specific designation (in the genera *Boweria*, *Crossothea*, *Renaultia*, and *Sphenopteris*) and they are separable chiefly by their fructifications (which are, however, rarely found in organic connexion with typical sterile parts of the frond). The name *Crossothea kidstoni* is therefore desirable as referring to typical specimens of a very variable frond.

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EXPLANATION OF PLATES XXXIII AND XXXIV.

Illustrating Dr. Crookall's paper on *Crossotheca* and *Lyginopteris oldhamia*.

PLATE XXXIII.

- Fig. 1. *Sphenopteris hoeninghausi*, Brongniart (approx. $\times 1$).
Copy of part of Brongniart's figure of his type specimen, representing the form of foliage with rounded lobes.
- Fig. 2. *Sphenopteris hoeninghausi*, Brongniart (approx. $\times 1$).
Copy of part of Andrae's figure of *S. hoeninghausi* illustrating the cuneate type of foliage.
- Fig. 3. *Sphenopteris hoeninghausi*, Brongniart ($\times 2$).
Horizon : Ten-foot Ironstone Measures (Yorkian Series).
Locality : Claycroft Openworks, Coseley, near Dudley, South Staffordshire.
Photograph by R. Kidston.
Fragment of pinnae comparable with Brongniart's figure (here reproduced at Fig. 1).
Collection of H. W. Hughes.
- Fig. 4. *Sphenopteris hoeninghausi*, Brongniart ($\times 2$).
Horizon and locality as above.
Photograph by R. Kidston.
Leaflets intermediate between those shown at Figs. 3 and 7.
Collection of H. W. Hughes.
- Figs. 5, 6. *Sphenopteris hoeninghausi*, Brongniart ($\times 1 \frac{1}{6}$).
Horizon and locality as above.
Pinnules of an almost linear type, approaching *Crossotheca schatzlarensis* in this respect.
Collection of H. W. Hughes.
- Fig. 7. *Sphenopteris hoeninghausi*, Brongniart ($\times 2$).
Horizon and locality as above.
Photograph by R. Kidston.
Cuneate type of foliage comparable with that figured by Andrae (refigured at Fig. 2 above).
Collection of H. W. Hughes.

PLATE XXXIV.

- Fig. 8. *Sphenopteris hoeninghausi*, Brongniart ($\times 1 \frac{1}{6}$).
Horizon : roof of Fireclay Coal (Yorkian Series).
Locality : Doulton's Marl Quarry, Netherton, South Staffs.
Collected by H. W. Hughes, Kidston Coll., No. 939.
Specimen illustrates an example of *S. hoeninghausi* of Yorkian age.
- Figs. 9 and 19. *Sphenopteris hoeninghausi*, Brongniart, Fig. 9 ($\times 1 \frac{1}{6}$); Fig. 19 ($\times 4$).
Fig. 9. Horizon : between Fireclay and Bottom Coals (Yorkian Series).
Locality : as above.
Collected by H. W. Hughes, Kidston Coll., No. 940.
Another example of the species from Yorkian rocks.
Fig. 19 (by R. Kidston) shows spines on rachis and pinnule segments.

Fig. 10. *Sphenopteris hoeninghausi*, Brongniart ($\times 1 \frac{1}{3}$).

Horizon: Ten-foot Ironstone Measures (Yorkian Series).

Locality: Clayscroft Openworks, Coseley, Dudley.

Collection of H. W. Hughes.

Lower pinnules tend to be lobed, upper to the cuneate type of foliage. A form intermediate between those shown on Plate XXXIII, Figs. 3 and 4.

Figs. 11 and 12. *Sphenopteris hoeninghausi*, Brongniart ($\times 1 \frac{1}{3}$).

Horizon and locality as above.

Collection: British Museum (Natural History), No. V, 1373.

Differences are exhibited between the two halves of the nodule: compare especially the portions indicated by arrows. These differences are merely due to the splitting of the nodule.

Figs. 13 and 20. *Sphenopteris hoeninghausi*, Brongniart, Fig. 13 ($\times 1 \frac{3}{4}$); Fig. 20 ($\times 2$).

Horizon: Three-quarter Coal (Yorkian Series).

Locality: Chopwell, $2\frac{1}{2}$ miles N.E. of Ebchester, co. Durham.

Collected by P. Charlton, Kidston Coll., No. 5174.

Specimen of *S. hoeninghausi* of Yorkian age.

Fig. 14. *Sphenopteris hoeninghausi*, Brongniart ($\times 1 \frac{1}{3}$).

Horizon: Ten-foot Ironstone Measures (Yorkian Series).

Locality: Clayscroft Openworks, Coseley, Dudley.

Collected by H. W. Hughes, Kidston Coll., No. 942.

Cuneate type of leaf: effect probably exaggerated by loss of lamina on other half of nodule (which is not available for comparison).

Fig. 15. *Crossothea kidstoni* ($\times 1$).

Horizon and locality as above.

Collection of H. W. Hughes.

Specimen shows (at base) sterile foliage of the cuneate type referable to *S. hoeninghausi* and fertile pinnules of *C. kidstoni* in organic connexion. Photograph by R. Kidston.

Fig. 16. *Crossothea kidstoni* ($\times 2$).

Horizon and locality as above.

Collection of H. W. Hughes.

Portion of a pinna of *C. kidstoni*. Photograph by R. Kidston.

Fig. 17. *Crossothea kidstoni* ($\times 2$).

Horizon and locality as above.

Collection of H. W. Hughes.

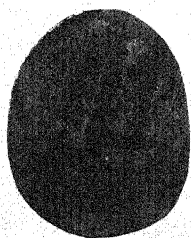
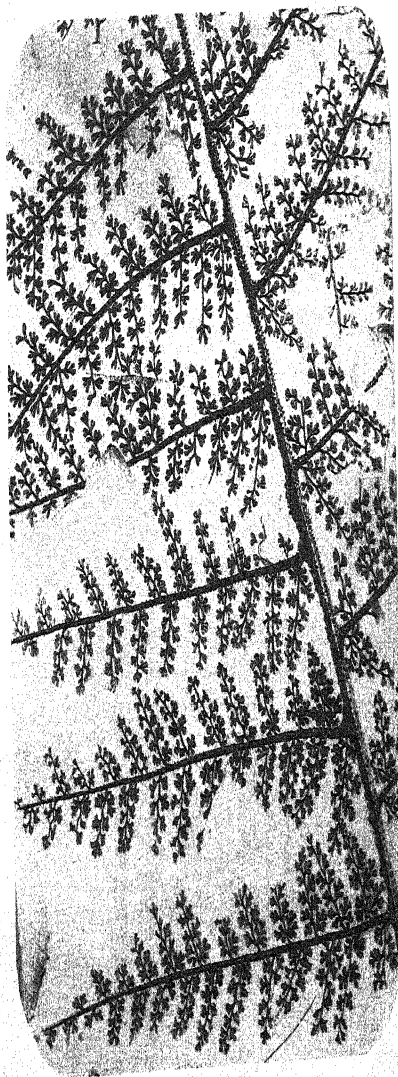
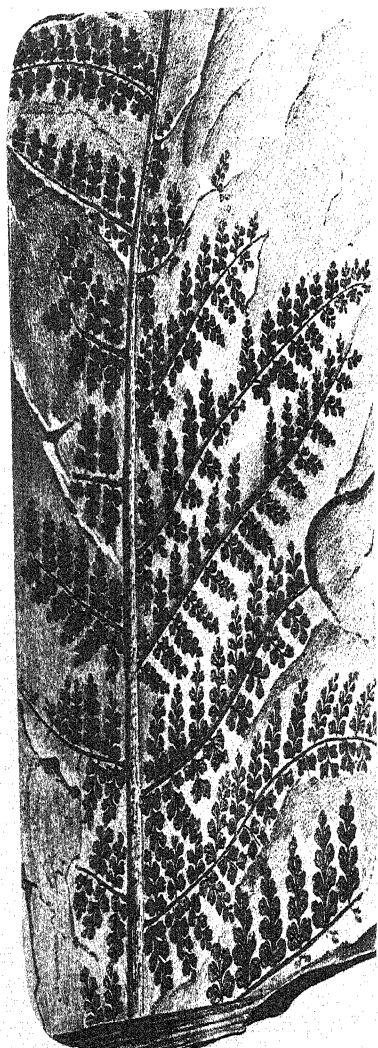
Portion of a pinna of *C. kidstoni* showing reticulate cortex at the base of the main rachis.

Fig. 18. *Sphenopteris hoeninghausi*, Brongniart ($\times 1 \frac{1}{7}$).

Horizon and locality as above.

Collection of H. W. Hughes.

Cuneate type of foliage: effect probably increased by splitting of nodule (compare the two portions marked by arrows).





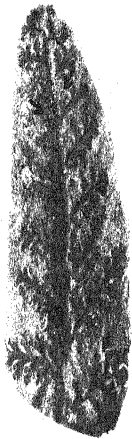
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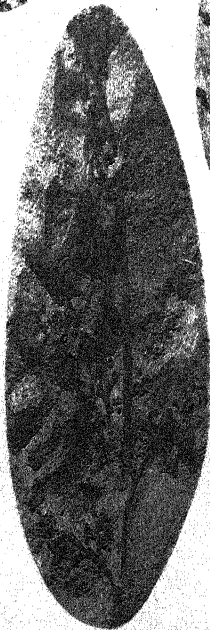
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10



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15



20



9

Seed Dispersal from the Hygroscopic Fruits of *Mesembryanthemum Carpanthea* (*Mesembryanthemum*), *pomeridiana* N. E. Br.

BY

S. GARSIDE

AND

S. LOCKYER.

With Plate XXXV and fifteen Figures in the Text.

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I. INTRODUCTION.

THE capsules of *Mesembryanthemum*, the valves of which open only when wetted, have long been objects of interest to botanists, though very little precise information, either of the anatomy of the capsule or of its mechanism, has been available.

In an early reference to the hygroscopic fruits of *Mesembryanthemum*, Steinbrinck (10) in 1883 suggested that the capsule valves protect the seeds from the desiccating influence of the sun, as they remain closed except when wetted by rain. The boss projecting from the wall of the ovary and partially closing the aperture of each compartment in *M. linguaciforme*, Haw. he regards as an additional device to prevent the seeds from escaping too readily from the protecting capsule.

Kerner and Oliver (8) in 1895 gave two figures of the fruits of *Mesembryanthemum*. The first, '*M. annuum*', has the five-valved type of capsule

which, on opening, leaves the seeds completely exposed. The second capsule figured, *M. candolleum* (*Candollii*, Haw. = *pomeridianum*, L.), having 17 valves, is that of *Carpanthea pomeridiana*, N. E. Br., which forms the subject of this paper. Kerner merely remarks that the seeds are washed out by the rain.

More recent observations have been made by Berger (1). He allowed drops of water to fall from a height of 2 metres on to an open capsule, and obtained a radial distribution of seeds of 50 cm. Schmid (9), in an account of *M. pseudotruncatellum*, Berger. suggested that it is the weight of the falling drops of rain which ejects the seeds from their compartments, and distributes them in a circle in the zone covered by the water splashing from the capsule.

Recent work by N. E. Brown (3) led him to suggest that the structure of the capsule of *Mesembryanthemum* prevents the seeds from escaping too rapidly. In cases where membranous wings covering the loculi are absent, the seeds can, doubtless, be readily washed out by the rain, since they are uncovered when the capsule valves are open; where the loculi are covered in the open capsule, however, the escape of the seeds is more difficult to explain, and Brown regards the problem as being still unsolved, although he considers the washing out of the seeds by rain to be likely. This view agrees with that of Huber (7), who suggests that after the open capsule has become filled with water the seeds are torn away from the funiculi by the falling drops. On account of the flow of the water through the inner chambers of the capsule some seeds are washed on to the upper side of the open fruit, from whence they are splashed away by the falling drops of water.

H. M. L. Bolus (2) is probably of the same opinion as N. E. Brown, i.e. that the seeds do not escape readily, for she describes a case where two young plants of *Carpanthea pomeridiana*, N. E. Br. were found flowering but still attached to the capsule, the seeds having germinated *in situ* in the loculi. The capsule of *C. pomeridiana*, N. E. Br. has been used in the present investigation of the anatomy and seed dispersal mechanism. It is one of the most complicated types of fruit, and a casual examination gives the impression that it is almost impossible for the seeds to escape, except by decay of the capsule wall—a condition which might have arisen owing to over-specialization. However, a critical examination in the laboratory, using carefully devised experimental methods, has shown this capsule to be a most efficient mechanism for the dispersal of the seeds by rain.

II. DESCRIPTION OF PLANT.

Brown's (4) observations on the fruits and flowers of *Mesembryanthemum* led him to split up the very large genus into a number of new genera, of which the monotypic genus *Carpanthea* is one.

Carpanthea pomeridiana, N.E. Br. is a freely-flowering annual which occurs on sandy areas and cultivated lands on the Cape Peninsula and adjoining mainland. The leaves, which are mostly radical, are opposite, flat, and somewhat succulent. The large (5.5 cm. diameter) lemon-yellow coloured flowers are born on erect pedicels which are much longer than the leaves, but which become prostrate in the fruiting stage. The green, five-lobed perianth ('calyx') surrounds and supports several rows of very numerous slender lemon-yellow staminodes ('petals'), within which are several rows of yellow stamens, the outer stamens being filiform and without anthers. In the centre of the flower are 10-20 threadlike pointed stigmas, the number being the same as the number of loculi in the large inferior ovary. The stamens are at first inflexed to the centre of the flower, and cover the stigmas; later they spread outwards and expose the latter. The ovary, which is much broader than deep, increases considerably in size after fertilization, and matures to form a capsule with 10 to 20 valves and as many loculi. The details of the structure of this fruit will be given later. Each loculus contains from 5 to 7 D-shaped black seeds, with minutely tuberculated testa.

III. MORPHOLOGY OF THE FRUIT.

The number of valves in the fruits of *Carpanthea* is very variable, as can be seen from the following table, which gives the figures for 177 fruits that were examined:

Variation in No. of Valves of Fruit.

No. of valves	10	11	12	13	14	15	16	17	18	19
No. of fruits with above No. of valves	4	5	22	22	25	41	26	22	4	6

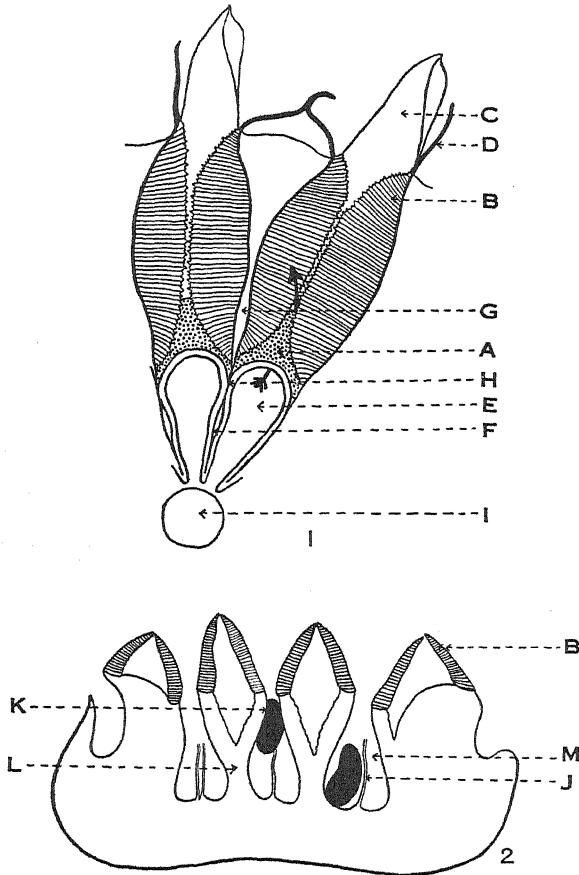
The table shows that fruits with 15 valves are the most common. There is no correlation between the size of the fruit and the number of the valves.

When the fruit is mature and dry the withered remains of the calyx are visible round the outside of the fruit, and remains of stigmas are often to be seen on the tips of the valves (Pl. XXXV, Fig. 1). The valves do not correspond to carpels, although they equal these in number, for each rests over a septum and not over a loculus; hence the halves of each valve are formed from adjacent carpels.

Pl. XXXV, Fig. 2, shows the same fruit after it has been expanded in water. Each valve (Text-fig. 1 C) has opened outwards, exposing on its underside two fan-shaped keels (B) ending in long awns (D). Each keel is attached to the top of a septum between two loculi, starting approximately midway between the central axis of the fruit and its outer wall, and runs from thence outwards along the underside of a valve for about half its length, after which it is prolonged to form a free awn. The two keels of each valve are

attached some distance apart, but their inner faces converge, so that the free edges are in contact for part of their length (Text-fig. 1).

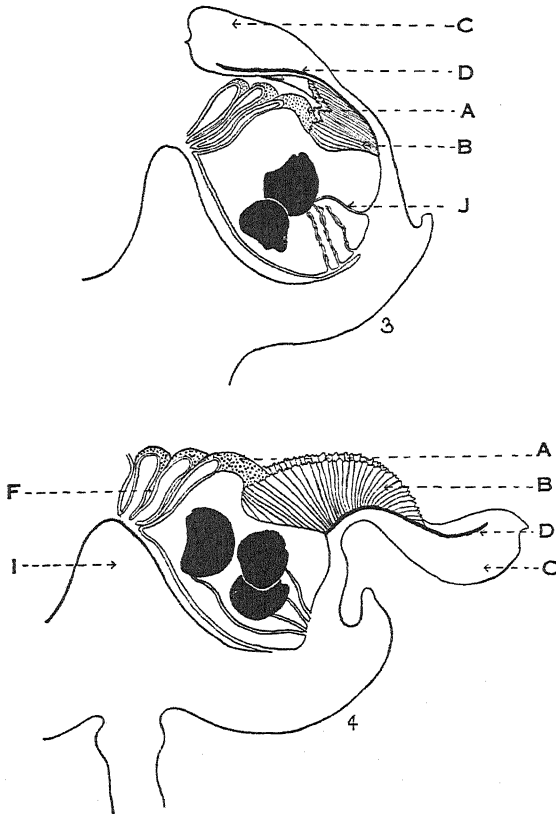
The seeds are not easily seen when the fruit opens, for each septum (Text-fig. 2 L) is split vertically, and the halves, which are of a cartilaginous nature, diverge and so arch over the loculi (M).



TEXT-FIGS. 1 and 2. 1. Surface view of an open fruit showing two valves. A. Horny arch which keeps the two halves of the septum apart. B. Hygroscopic keel. C. Valve. D. Awn. E. Space between the two halves of a split septum. F. Space above loculus, towards the centre of the capsule, through which the seeds are ejected. G. Space above loculus between the bases of the valves. H. Point where the septa are forced together above the loculus. I. Central column. 2. Tangential section of open fruit. J. Funiculus. K. Seed. L. Septum. M. Loculus. $\times 5$.

The septa are split to the base towards the centre of the fruit, but only through about half their height, where they join the ovary wall. As the two keels of each pair are attached to the two halves of each septum they are necessarily some distance apart at their attached edges (Text-fig. 2). The halves of each septum are joined by a narrow arch (Text-fig. 1 A) situated approximately midway between the centre of the fruit and the ovary wall.

In the figure an arrow has been drawn passing under this. The arch is of a horny texture and serves to keep the two halves of each septum apart, so that they diverge and arch over the loculi. The elasticity of the arch forces



TEXT-FIGS. 3 and 4. 3. Vertical and radial section through closed fruit, showing a loculus in section. 4. Vertical and radial section through open fruit. Lettering as in Text-figs. 1 and 2. $\times 5$.

the septa to meet above the loculus (H), but a narrow space is left towards the centre of the capsule (F) and between the bases of the valves (G). Seeds may often be seen through the former opening (F).

As the loculi have no roofs (Text-fig. 2) the seeds would be completely exposed when the valves are reflexed (Text-fig. 1) were it not for the fact that the split septa arch over the loculi (Text-fig. 2 M). Although there is a narrow opening above each loculus (Text-fig. 1 F) the aperture is not wide enough to allow the seeds to escape unless force is used, as can easily be seen if one attempts to displace the seeds with a needle.

Brown found that when capsules of certain types were repeatedly opened by wetting, on each occasion a few seeds fell out of them into the saucer in which they were placed. He suggests that probably, under

natural conditions, a few seeds only are liberated each time the capsule opens; this he considers to be an adaptation to the dry climate, 'for if all the seeds were shed at once and the subsequent rainfall insufficient to enable the seedlings to establish themselves the effort of the plant would be wasted for that year' (Brown (3), p. 173).

If a closed fruit be cut vertically and radially between two valves, a loculus is seen in section (Text-fig. 3). When the fruit is mature the hardened walls of the carpels separate from the central conical axis or columella of the fruit (Pl. XXXV, Figs. 1 and 4), and also to a great extent from each other, because the septa are split to their bases in the region of the columella.

The free follicle-like tip of each carpel rests upon the corky columella, and the base of each carpel is also to a great extent detached from the corky floor of the ovary. The placenta is situated on the floor of the carpel towards the periphery of the fruit; and attached to it are numerous very long funiculi, often twisted together, some bearing ripe seeds, others unfertilized withered ovules. As many as seven ripe seeds may occur in a single loculus. The funiculi are so delicate that the relatively heavy seeds are easily broken away, and it is scarcely possible to remove a seed with its funiculus attached.

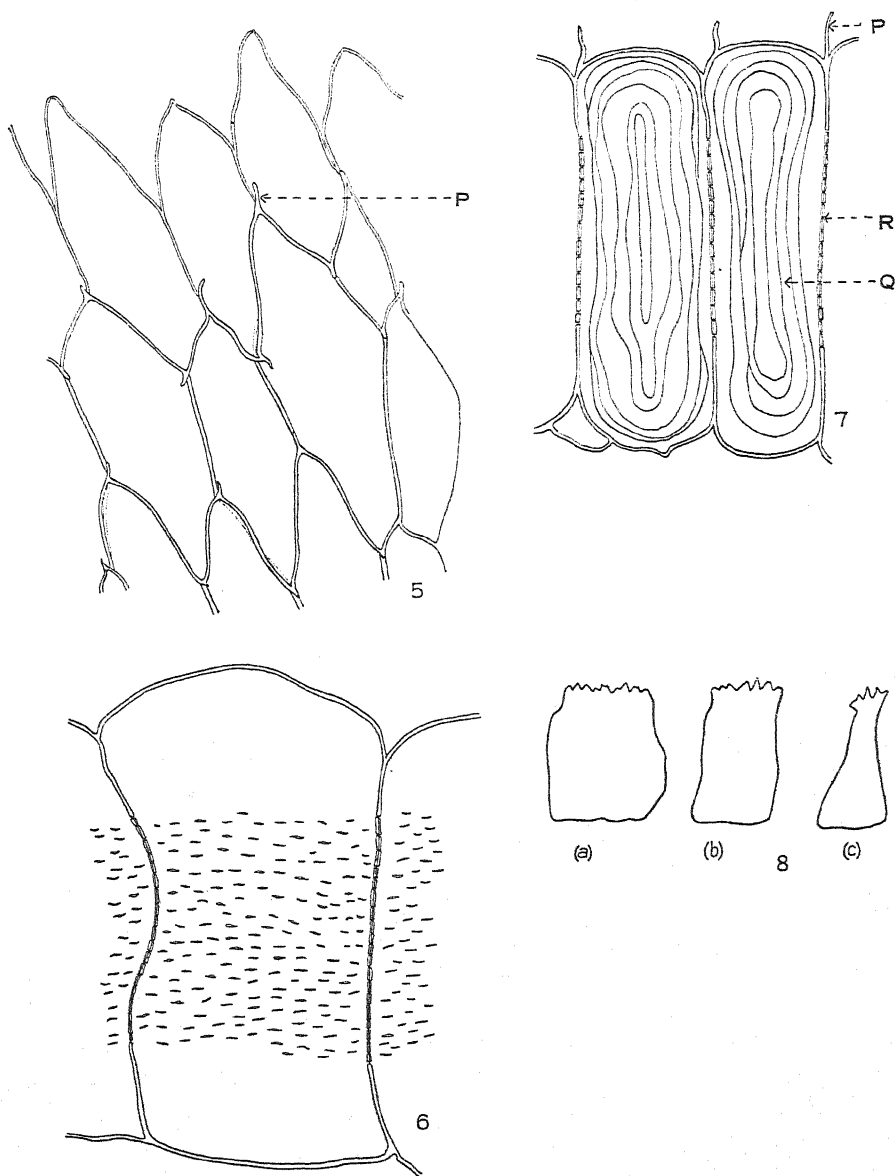
In the dry fruit the keels are hard and woody and compactly folded under the closed valve (Text-fig. 3). The arch (A) joining the halves of the septum extends just beyond the point of attachment of the keels.

When the fruit is wetted the keels absorb water and expand rapidly. They unfold like a fan, and force the valve upwards and outwards until the fruit is fully open (Pl. XXXV, Fig. 2). On drying, the keels contract and the valves close again, but are ready to reopen under suitable conditions (Pl. XXXV, Fig. 1).

IV. STRUCTURE OF THE EXPANDING KEELS.

An expanding keel with its awn can be easily torn off from the valve and its structure examined after mounting in water.

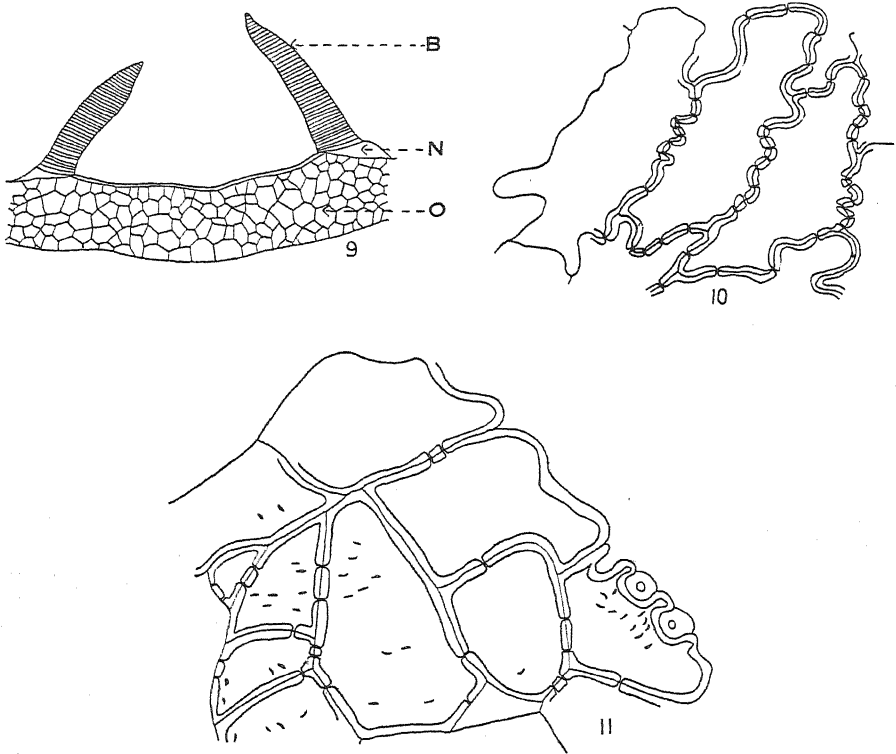
The keel is one cell in thickness (Text-fig. 9) except along the line of its attachment to the valve, where it is several cells thick (Text-fig. 11). The free edge of the keel is serrated, as the marginal cells have free tips which project irregularly (Text-fig. 5). In surface view the keel is seen to be composed of cells, the free surfaces of which are rhomboidal in shape, and arranged so that the long axis of each cell-face is parallel to a line joining the point of attachment of the keel to the free margin (Text-fig. 5). 150μ is an average length for the longer axis, the shorter axis being approximately 50μ . The cells therefore appear to radiate from a basal line of attachment and give the impression of an open fan.



TEXT-FIGS. 5-8. 5. Surface view of cells of hygroscopic keel in the fully expanded condition. P. Spine. $\times 250$. 6. Vertical section of a cell of a fully expanded keel, showing the pitted walls (the cell contents are omitted). $\times 250$. 7. Horizontal section of expanded keel. $\times 250$. R. Pits in the vertical walls. Q. Lamellated mucilage. 8. Outline drawings of the same piece of keel tissue in: (a) water; (b) 50 per cent. alcohol; (c) pure glycerine. $\times 15$.

The free surfaces of the cells are somewhat convex, and overlap slightly like the scales of a fish, the free tips of each cell being prolonged into a small spinous process (Text-fig. 5 P).

Each cell composing the keel, in the region in which it is one cell in



TEXT-FIGS. 9-11. 9. Transverse section of a valve to show attachment of the keels B. N. Specialized colourless basal tissue. O. Tissue of valve. $\times 15$. 10. Surface view of basal tissue of keel showing the irregularly lobed walls and well-marked pits. $\times 250$. 11. Vertical section through the specialized basal tissue of the keel. $\times 250$.

thickness, has the shape of an oblique prism, with two free rhomboidal faces (already described) and four faces in contact with adjoining cells. The latter faces are comparatively thin and lignified, and all are densely pitted over about half their area (Text-figs. 6 and 7), thus facilitating the rapid passage of water from cell to cell, whereas the two free rhomboidal faces are without pits. In addition to the lignified walls, every cell has a thick secondary deposit of lamellated mucilage entirely covering its inner face, and almost completely occluding the lumen, but with the remains of the protoplast still occupying the centre of the cell. The mucilage very readily imbibes water, thus causing the expansion of the keel. It becomes blue in colour when treated with chlor-zinc iodide, indicating that it is of a cellulose nature.

The anatomical structure of these cells is in agreement with that described by Von Guttenberg (6) for *Mesembryanthemum rhomboideum*, Salm-Dyck, and by Huber (7) for *M. linguaeforme*, Linn. The figures (Text-figs. 5, 6, 7) of cell structure of the keel were made from fully imbibed cells. When dry and contracted the tissue is thrown into folds and wrinkles, and it is difficult to distinguish individual cells. As a result, it is impossible to give comparative measurements of single cells in the dry and in the imbibed condition, but some idea of the degree of expansion may be obtained by comparing the width of a piece of keel tissue in the dry and in the imbibed state. Text-fig. 8 illustrates the comparative sizes of a piece of keel mounted in (a) water, (b) 50 per cent. alcohol, and (c) pure glycerine. In the fully imbibed condition the keel tissue is approximately four times the length of the same piece when dry. It can be seen from the figure that increase in size takes place almost entirely in a direction parallel to the margin of the keel, i.e. in the best direction for bringing about the opening of the capsule valves.

Each of the two keels is separately attached to the valve by a specialized colourless basal tissue which is several cells in thickness (Text-fig. 9 N). The keel cells resting upon this colourless tissue are smaller, with thicker walls, and are darker brown in colour than the normal cells of the keel. Towards the apex of the valve these modified cells of the keel are prolonged to form a free awn composed of very elongated cells of the same type.

The superficial cells of the basal region are irregularly lobed, and their colourless thick walls have well-marked pits (Text-fig. 10). Below this layer the more deeply seated cells are less lobed, but have similar pits (Text-fig. 11).

V. THE SEED.

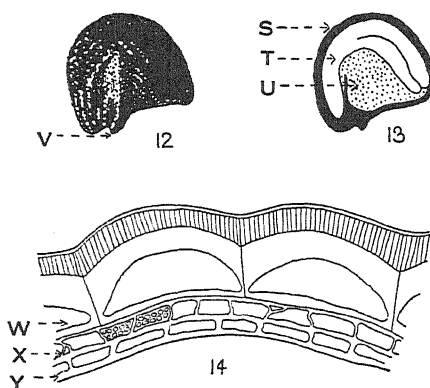
The campylotropous seeds are D-shaped and compressed, with a rough, very resistant testa (Pl. XXXV, Fig. 3). There is no obvious external trace of the micropyle, but the seed shows a tubercle on its straight margin which marks the point of attachment of the funiculus (Text-fig. 12 V).

The oil-containing embryo (Text-fig. 13 T) is curved round a starch containing parenchyma (U), interpreted by Huber (7) as perisperm. In section the epidermis of the seed consists of cells approximately 85μ long by 50μ high, whose outer walls are strongly convex. This outer wall of the epidermal cell is three-layered; externally there is a thin colourless cuticular skin, then a dark brown region approximately 12μ thick, while the innermost yellowish brown layer is also 12μ thick. The lumen of the cell is completely filled with a reddish brown substance.

The sub-epidermal layer (Text-fig. 14 X) is composed of rectangular cells in section 24μ long by 10μ high. These cells are completely filled with small crystals of calcium oxalate. The third layer shows cells of

similar size and form, but their walls are thicker and their contents dark brown in colour.

The walls of all three layers are strongly suberized and are unaffected



TEXT-FIGS. 12-14. 12. Surface view of seed showing its rough testa. v. Tubercle which marks the point of attachment of the funiculus. $\times 7.5$. 13. Vertical section through the seed. s. Testa. t. Curved embryo. u. Starch containing parenchyma. $\times 7.5$. 14. Section of testa. w. Epidermis of testa. x. Sub-epidermis with cells containing crystals of calcium oxalate. y. Third layer of testa. $\times 250$.

by concentrated sulphuric acid; their colour suggests them to be strongly impregnated with tannin.

According to Huber (7) the two outer layers of the testa are derived from the outer integument, while the third layer is derived from the inner integument.

VI. SEED DISPERSAL BY ARTIFICIAL RAIN.

A knowledge of the structure of the fruit of *Carpanthea* gives little or no indication as to the mode of seed dispersal. In fact, a more intimate knowledge of the morphology of the fruit completely justifies Brown's statement that 'they would seem to be specially designed to prevent the seed from escaping' (Brown (3), p. 151). Experiments were therefore made to determine whether falling water drops would cause ejection of the seeds.

Experiment I.

A dry capsule was fixed by its stalk into a small dish of paraffin wax, and drops of water were allowed to fall on to it from a height of 1.75 m. The capsule valves opened, and as the drops struck the interior of the capsule seeds were forced out and carried to a distance by the splashing water. Each seed was ejected so quickly that it was not possible to tell whether it was forced from the loculus through slit F (Text-fig. 1), or whether the drops falling on the centre forced the seeds out laterally

through slit G. The drop of water which displaces the seed from its locus also, on splashing, carries the seed to some distance from the capsule. All the seeds (about 30) were dispersed within 20 minutes of the drops beginning to fall. Each locus was afterwards explored with the point of a needle in order to make certain that the capsule had been completely emptied of seeds. This experiment shows in a simple way that drops of water falling from a height are most efficient in seed ejection.

Experiment II.

A capsule was opened by soaking in water, so that its seeds were not displaced, and after the surface water had been removed melted wax was run into the central depression of the fruit, around and above the columella (Text-fig. 1). This has the effect of closing those openings of the follicles (F) which are directed towards the centre of the fruit.

In this particular capsule nine seeds were visible lodged at the outer openings of the follicles when the fruit was first opened. When the open capsule was put under the falling water drops these nine seeds were immediately splashed out, but although the experiment was continued 20 minutes no other seeds were removed. On removal of the wax, however, seeds immediately began to come out, and the capsule was completely empty in 26 minutes from the time that the inner openings to the follicles had been freed. This result indicated that the seeds normally escape towards the centre of the capsule, and a further experiment was therefore devised to prove this.

Experiment III.

A small circle of transparent celluloid was cut to form a cover fitting exactly the central depression of a fruit. This cover was held in place, over the depression, by a pin passing through its centre and inserted in the columella. A small piece of sealing wax was applied to prevent the celluloid cover from slipping down the pin. The cover was adjusted so that sufficient room was left for the seeds to escape from their carpels into the cup-like centre of the fruit through the slits at F, but it prevented the ejected seeds from being carried away by splashing water drops.

A capsule treated in this manner was placed under the dripping water. After three minutes the cover was removed and many ejected seeds were found lodged underneath it.

These experiments (II and III) lead to the conclusion that the seeds are forced out through the slits opening towards the centre of the fruit. A large drop, falling with some force into the locus through the opening G (Text-fig. 1), drives the seeds through the valve-like aperture F and ejects them towards the centre of the capsule.

Experiment IV.

The height from which the drops fell was varied in order to see whether there was a limiting height below which the energy of the falling drop was insufficient to bring out the seeds, i.e. to force them through the elastic valve-like aperture F. No exact limit was found, for drops falling from only 0.5 m. could eject one or two seeds from a capsule. It is obvious, however, that there may be a few seeds loosely placed near the openings of the follicles, and these would need far less energy to disperse them than those more firmly lodged at the base of the compartments. For each height a few seeds were displaced, but only when the drops fell from a height of 1.75 m. or more was the mechanism really effective. With drops falling from this height four seeds were shot to a distance of over 1 m. With drops falling from 2 m. the farthest distance a seed was observed to travel was 0.88 m. It is interesting to note here that Berger (1), when allowing drops to fall from a height of 2 m., obtained a radial distribution of 0.5 m., and Huber obtained a distribution of over 1 m., but these distances were obtained with other types of fruits.

Dissection of the capsule which had been used for the above tests showed three seeds still lodged at the bases of the follicles. It is probable that in nature two or three seeds in each capsule may fail to be ejected by rain in much the same way. They may be so tightly lodged at the base of the deep follicles that the falling rain has insufficient energy to move them. Probably it was seeds such as these that gave rise to the two plants that Bolus (2) found flowering but still attached by their roots to a capsule. Although this may happen as an exception, the experiments already described show that the fruit of *Carpanthea pomeridiana* is an extraordinarily effective mechanism for seed dispersal.

These experiments also disprove the idea that only one or two seeds are dispersed each time the capsule opens, as Brown (3) suggested. Drops falling at the rate of 20 a minute will open a capsule and disperse all its seeds in 20 minutes, so that a heavy thunderstorm could accomplish this in a much shorter time. It is obvious that a fine or drizzly rain will be ineffective, for although it will cause the capsule to open it will not necessarily disperse its seeds.

Although a very few drops of water falling directly on to one of these capsules will bring about its opening, a saturated atmosphere, even when maintained for three or four hours, has been shown experimentally to be unable to bring about any opening.

Some calculations have been made in connexion with the above experiments. In the laboratory drops of 0.269 cm. radius falling from a height of 175 cm. were effective for seed dispersal. By means of these data the limiting velocity of a drop of this size was calculated, and also its

velocity after falling through a distance of 175 cm. Hence, knowing the mass and velocity of a drop which had been shown experimentally to be effective for seed dispersal, the smallest drop capable of bringing about the same effect, when travelling at its limiting velocity, was calculated (on the assumption that the energy of the drop is the determining factor in the ejection of the seeds). Actually the viscosity of the air is so small that it is unlikely that any drops attain their limiting velocity before reaching the earth. Consequently the smallest drop which would be effective for seed dispersal is larger than that calculated.

The following data were used in the calculation :

v_0 = limiting velocity of experimental drop.

r = radius of experimental drop = 0.269 cm.

m = mass of experimental drop = 0.0813 grm.

g = acceleration due to gravity = 982.

η = viscosity of air = 181×10^{-6} .

s = distance fallen by experimental drop = 175 cm.

v = velocity attained by experimental drop after falling distance of 175 cm.

R = radius of smallest drop capable of producing same result when falling at its limiting velocity.

m' = mass of drop of radius R .

v_0' = limiting velocity of drop of radius R .

ρ = density of water.

σ = density of air.

Stoke's formula was applied to determine the limiting velocity of the experimental drop, and in so doing $(\rho - \sigma)$ was taken as unity, so that

$$\begin{aligned} (1) \quad v_0 &= \frac{2}{9} g \frac{r^2}{\eta} (\rho - \sigma) \\ &= \frac{2}{9} g \frac{r^2}{\eta} \\ &= \frac{2}{9} \times \frac{0.269^2 \times 982}{181 \times 10^{-6}} \\ &= 87,260 \text{ cm. per sec.} \end{aligned}$$

As η is very small we may get the velocity of the experimental drops by

$$\begin{aligned} (2) \quad v^2 &= 2gs; \\ \therefore v &= 586.2. \end{aligned}$$

A more accurate formula taking viscosity into account is

$$\begin{aligned} (3) \quad \frac{gs}{v_0^2} &= \log \frac{1}{\Sigma} + \Sigma - 1, \\ \text{where} \quad \Sigma &= \frac{v_0 - v}{v_0}, \end{aligned}$$

deduced on the assumption that the mass of the drop is all the mass in motion, i.e. neglecting the mass of the air carried along with the drop.

$$\frac{gs}{v_0^2} = \frac{(1-\Sigma)^2}{2}, \text{ neglecting higher powers of } (1-\Sigma)$$

$$\sqrt{\frac{gs}{v_0^2}} \times 2 = 1 - \Sigma$$

$$\frac{982 \times 175 \times 2}{87260^2} = 1 - \Sigma$$

$$.006719 = 1 - \Sigma$$

$$\frac{v_0 - v}{v_0} = \Sigma$$

$$1 - \Sigma = \frac{v}{v_0}$$

$$v = .006719 \times 87260$$

$$v = 586.2,$$

which agrees with the result obtained with formula (2), and therefore shows that the viscosity of the air is a negligible factor when dealing with such a short distance. Then, since

$$mv^2 = m'v'^2$$

$$= \frac{4}{3} \pi R^3 \left(\frac{2}{9} \frac{R^2 g}{\eta} \right)^2$$

$$\therefore R = \sqrt[3]{\frac{mv^2}{g^2} \frac{243}{\pi} \frac{\eta^2}{16}}$$

$$= .06439 \text{ cm.}$$

Meteorologists (5) divide raindrops into the three following classes :

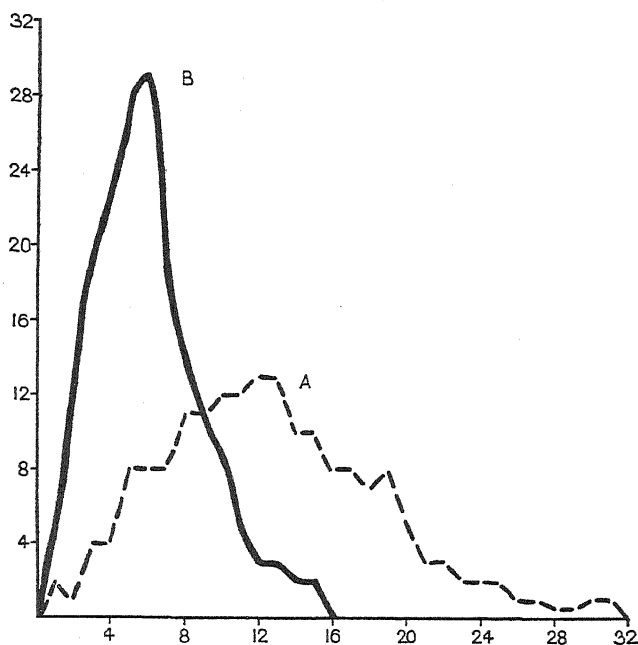
Fine	rain	.	.	.	0.0125 cm. radius
Medium	"	.	.	.	0.15 " "
Heavy	"	.	.	.	0.3 " "

According to the calculation made above, 0.0644 cm. radius is the smallest size drop that will be effective. Comparing the effective size of raindrop, found by calculation, with the above figures, we find that it accords with the suggestion made previously, namely, that 'a fine or drizzly rain will be ineffective, for although it will cause the capsule to open it will not necessarily disperse its seeds'. Thus 'medium' rain, if falling from a great height, or 'heavy' rain, falling only a short distance, will bring about ejection of the seeds from the capsules.

VII. RATE OF EXPANSION OF HYGROSCOPIC KEELS.

Among other experiments designed to throw light on the nature of the valve mechanism were some in which the rate of movement of the

valves was studied. For this purpose capsules were cut up and portions of carpels and attached valve and keels used separately. A fine capillary glass pointer was attached to the tip of a valve and the tissues fixed above



TEXT-FIG. 15. Graph of the rate of movement of a valve: A At 10.5°C; B Same valve at 37°C. Abscissae-time in half-minutes; ordinate degrees of angle.

a protractor by means of a pin inserted through the base of the septa. Wax was used to prevent the valve from slipping round on the pin. The dry valve was arranged so that the glass pointer lay along the 0° line. The apparatus was then covered with water, when the keel expanded and rotated the valve so that the pointer passed over the scale until it came to rest approximately over the 180° line. Most valves were found to move through 180°, although variations slightly above or below this value occurred.

The angle through which the pointer moved was measured every half minute, and in this way the rates for different valves were compared. Valves from the same capsule were found to behave in like manner, but differences were found in the behaviour of valves from different capsules. As a general rule the rate of movement increases until an approximately vertical position is reached, after which a gradual slowing off occurs until about 175°. After this point the rate is often so slow as to be almost imperceptible.

The rate of movement was found to increase with rise of temperature. Text-fig. 15 shows a graph illustrating the behaviour of the same valve at different temperatures. At 10.5°C. the valve took 16 mins. to move through 180°, while at 37°C. the time taken was only 7.5 mins. At high

temperatures the rate is not only higher but is more uniform than at lower temperatures ; a graph of the former shows a smooth steep curve, while a curve of the latter is very irregular as well as being much flatter (Text-fig. 15).

Perhaps the most striking result of this investigation is the practical demonstration of the extent to which the capsule of *Carpanthea* is fitted for a type of seed dispersal brought about by falling rain. Almost all previous writers have commented upon the manner in which the seeds are enclosed by the carpels, and have suggested a slow and gradual emptying of the capsule by successive rainstorms, or even liberation of seeds by decay of the capsule wall. If it is borne in mind that the plant is an annual, it will be readily seen that such a slow mode of dispersal would not be favourable. The plant, represented only by seeds during the dry Cape summer, must take full advantage of the rainy winter season for its vegetative and reproductive activities ; and sufficient seed for the continuation of the species must be formed before the dry season again approaches. As the capsule matures the peduncle becomes prostrate, bringing the broad flat base of the capsule to rest upon the ground, the capsule valves thus being directed upwards. Usually the capsule remains in this position, attached to the withered remains of the parent plant, and it is only thus that it can function properly. If the capsules became detached and blown about by the wind, many of them would settle with the upper portion of the capsule towards the soil, in which case the seeds could not escape. Normally, however, the first rainstorms find the capsules still favourably orientated, with their broad bases firmly bedded on the soil, and their valves directed towards the clouds.

In this position the whole energy of the falling drops is available for seed ejection, for the capsules being on a firm basis do not give way or become knocked over by the force of impact, and they therefore stand every chance of becoming completely empty in a few minutes.

SUMMARY.

1. The *Carpanthea* fruit has from 10 to 20 carpels, 15 being the most frequent number.
2. The valves equal the carpels in number and are over the septa, not over the loculi.
3. The septa are split, the halves diverging and so roofing the loculi, so that the seeds are almost invisible when the valves are open.
4. Two slits are left, one between each pair of valves, and one towards the central axis of the fruit. Experiments show that the seeds escape by the latter aperture only.
5. All the seeds in a capsule are readily dispersed in a short time (10 to 20 minutes) by falling raindrops, contrary to previous supposition.
6. Radial distribution of seeds, to about 1 metre from the capsule, is

caused by drops of water approximately the size of the drops falling during heavy rain.

7. A calculation has been made to determine the size of the smallest raindrop in nature capable of causing efficient ejection. This was found to be a drop of 0.06 cm. radius, and corresponds to 'medium' rain in nature. Fine rain fails to eject seeds, but causes the capsule to open. A dry capsule remains closed in damp air.

8. Each capsule valve has two hygroscopic keels composed of mucilage-containing cells, which imbibe water readily, and thus bring about the rapid opening of the valve. The time required for the complete opening of the valves decreases with rise of temperature.

9. The rate of opening of the valve increases until the valve is half open; beyond this the rate decreases until the fully opened condition is attained.

The material used for this investigation was collected from Stellenbosch Flats, South Africa, in December, 1920, and from Somerset West C.P., December, 1929.

In conclusion, we wish to thank Prof. W. Wilson, of Bedford College, for help with the mathematical section, and Mr. N. E. Brown for identification of the material used.

BOTANICAL DEPARTMENT,
BEDFORD COLLEGE.
May, 1930.

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EXPLANATION OF PLATE XXXV.

Illustrating S. Garside and S. Lockyer's paper on Seed-dispersal from the Hygroscopic Fruits of *Carpanthea* (*Mesembryanthemum*) *pomeridiana*, N.E.Br.

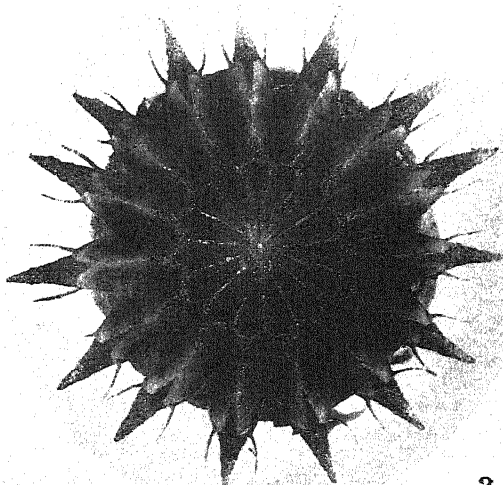
Fig. 1. × Closed fruit.

Fig. 2. Open fruit.

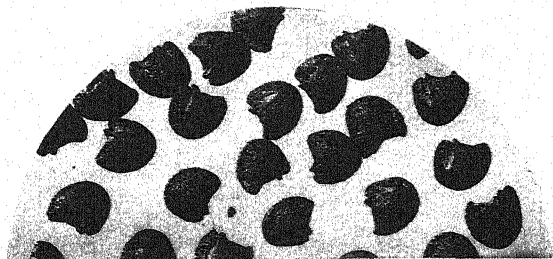
Fig. 3. Seeds.



1



2



3

GARSDALE AND LOCKYER — SEED DISPERSAL.

Huth. London.

Studies on the Transport of Nitrogenous Substances in the Cotton Plant.¹

V. Movement to the Boll.

BY

E. J. MASKELL

AND

T. G. MASON.

With seven Figures in the Text.

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SECTION I. INTRODUCTION.

ON the basis of work already reported (5, 6, 7, 8, 11), we have suggested that there is a gradient basis for the transport of nitrogen as well as for carbohydrates. In the case of carbohydrates the head in the leaf appears to be reducing sugars. Longitudinal movement through the sieve-tubes takes place mainly in the form of sucrose, but reducing sugars also doubtless take some part in carbohydrate translocation. In fact, if the acceleration of

¹ Paper No. 7 from the Physiological Department of the Cotton Research Station, Trinidad.

diffusion along the phloem is due to protoplasmic streaming of the contents of the sieve-tube, both sugars should play a part, the part played by each depending only on its concentration gradient and its diffusion constant. Movement from the sieve-tube into the boll seems to occur only in the form of sucrose. For nitrogen the head in the leaf appears to be residual N.¹ Within the sieve-tubes it seems possible that all the fractions may share in longitudinal movement, the part played by each depending on the dynamic concentration gradient which each maintains, also probably on its diffusion constant. Obviously in the case of protein only the non-structural protein could contribute to movement, and even in the case of the crystalloid fractions the concentrations determined in the expressed sap may include a storage component that does not contribute to movement in the sieve-tubes. Movement from the sieve-tubes into other tissues and vice versa must presumably be confined to crystalloid N, and there is some evidence that residual N may be the most important fraction. The scheme suggested for nitrogen transport is, of course, a very tentative one. Much of the difficulty in interpreting the phenomena of nitrogen transport appears to be due to the ease with which one fraction is converted into another. In many cases this must involve conversion of translocatory into storage nitrogen and vice versa. In addition translocatory nitrogen seems to escape from the sieve-tubes into the adjacent tissues with considerable ease; both factors should tend to damp the spread of concentration changes, and thus make the response to treatment somewhat ill-defined. In this respect nitrogen and carbohydrate transport differ, for the transition from sugar to starch, and vice versa, in the bark seems to proceed relatively slowly.

The present paper deals with some observations on the uptake of nitrogen and carbohydrates by the fruit of the cotton plant, i. e. the boll, and is thus concerned, not only with the transport of materials, but also with their utilization. It is concerned, therefore, with the problems of transport and growth. On the one hand, we may hope to explain some of the phenomena of boll growth on the basis of what is known about transport; on the other hand, the observations on uptake may throw further light on the laws governing transport. Uptake by the boll involves longitudinal movement of material along the sieve-tubes of the bark and the pedicel, and also movement out of the sieve-tubes into the tissues of the boll. As indicated in an earlier paper (11), the phenomena of interchange between tissues may be more complex than the phenomena of movement between two regions in the same tissue. It seems reasonable, however, to suppose

¹ It should perhaps be emphasized that nothing is known as to the identity of residual N in the different organs of the plant. For the present we assume that approximately the same compounds are included in this fraction in leaf, bark, wood, and boll. It may be added that nothing is known either concerning the identity of amide N or amino N in different organs or in the same organ at different times.

that the laws determining direction and rate of movement between two tissues will be fundamentally similar to those determining direction and rate of movement along the main conducting tissues.

It was shown, for instance, in an earlier paper (11) that the rate of uptake of carbohydrate by the boll was much greater by day than by night, and that this variation in uptake was correlated with a diurnal variation in the gradient of sucrose concentration from bark to boll. Thus the phenomena of carbohydrate uptake by the boll fitted into the general picture of transport as determined by concentration gradients. In the same way the phenomena of nitrogen uptake by the boll may throw some light on the general problem of nitrogen transport.

We have already noted, in the case of nitrogen transport, that the spread of concentration changes along the bark may be subject to considerable damping; and in an earlier paper (5) we recorded our failure to detect any appreciable diurnal fluctuation in the bark of the main-axis of plants bearing bolls. We might expect, therefore, other things being equal, that diurnal fluctuations in uptake by the boll should be less marked for nitrogen than for carbohydrates. It seems certain, from some preliminary observations, that this is the case; for the ratio of total N to total dry weight of the growing boll is consistently higher at dawn than at dusk. The data are not, however, sufficiently complete to enable us to make an accurate comparison between the rates of nitrogen and of carbohydrate uptake by day and by night. This question is, therefore, left for a future paper, and two other aspects of boll growth will be discussed here.

In the first experiment we study the effect of removing the bolls on the concentrations of different nitrogen compounds in leaf and stem tissues. In the second we attempt to discover the means by which the boll attracts nitrogen from the rest of the plant body. This we have done by determining the changes that take place in sap concentration and uptake of material during the first seven days of the growth of fertilized and unfertilized bolls.¹

SECTION 2. THE EFFECT OF REMOVAL OF THE BOLLS ON THE NITROGEN AND CARBOHYDRATE COMPOUNDS IN LEAF, BARK, AND WOOD. (EXPERIMENT 12.)

It was concluded, as a result of work done by one of us (9), some years ago in St. Vincent, that the retardation in the growth rate of the main axis of the cotton plant was due to a correlation factor introduced by the development of the bolls on the basal fruiting branches. It was suggested that the senescence of the main axis was due to a paucity of carbohydrates, and probably other growth-promoting substances, as a result of their

¹ The methods of chemical analysis used are described in detail in Part II of this series of papers (6).

diversion to the bolls. Essentially similar conclusions have been drawn by Murneek (12), except that he stresses the importance of nitrogen rather than of carbohydrates. Working on the tomato he found that 'the fruit diverts and monopolises in some way almost all the available nitrogen', and he traces the growth-retarding effect to this suction of nitrogen by the fruit. Murneek suggests that, under certain circumstances, for example, diminished photosynthetic activity, the drain on carbohydrate also might be severe enough to lead to a restriction of stem growth. Now both in the tomato and in the cotton plant the ratio of nitrogen to dry weight is very much greater in the fruit than in the vegetative parts of the plant. Consequently, unless the rates of elaboration of nitrogen and of carbohydrates by the leaves are in some way attuned to the requirements of the particular organs which are in process of growth, the removal of the growing fruits must lead to an increase of nitrogen relative to carbohydrates in the food material available for vegetative growth. An increase of nitrogen relative to dry weight, in the stem of plants from which fruits have been removed, would therefore be expected, and Murneek has shown that it does occur.

From the point of view of transport of food material the removal of the fruits may be regarded as the removal of a particular 'sink'; and the response shown by the elaborating and the conducting tissues should throw some light on the mechanism of transport. The case is in some respects parallel to ringing the main axis at ground level, for this operation isolates another 'sink', the root.

In the experiment to be described we determined, five days after removing the bolls, the changes in sap concentration, and in total amounts of material, that had taken place in selected leaves and in the bark and wood of two selected regions of the main axis.

(1) *Procedure.*

The plants were $14\frac{1}{2}$ weeks old, and were in the fifth week of flowering. The mean height was 95.7 cm., and the mean number of bolls per plant 15.3. Six samples, each of twenty-five plants, were drawn, the plants being graded by height and number of bolls. By means of wool, material for subsequent collections was marked as follows: (1) Upper region—the *stem* between the 12th and 7th nodes from the apex and the basal *leaf* on each of the three middle fruiting branches of this region. (2) Lower region—the *stem* between the 1st and 7th nodes, counting upwards from the lowest fruiting branch, and the basal *leaf* on each of the three middle fruiting branches of this region. On February 23, 1928, bolls, flowers, and flower buds were removed from three of the samples, forming the 'Bolls-off' group. The other three samples ('Bolls-on' group) were not touched. Five days later, material was taken for analysis. There were three collections—two in the morning and one in the afternoon. At each collection one of the Bolls-

off and one of the Bolls-on samples were taken. The stem samples were trimmed at the stipular marks and separated into bark and wood. Leaves, bark, and wood were weighed, cut up, and sub-samples taken in the usual way for drying and for freezing.

The determinations made on the sap were: crystalloid N, asparagine N, amino-acid N, nitrate N, ammonia N, sucrose, reducing sugars and hydrogen-ion concentration. Total N and reserve carbohydrates (polysaccharides) were determined on the dried material.

(2) Results.

(a) *Total amounts present.* The effect of removing the bolls on the total amounts of various substances in the internodes and leaves sampled is shown in Table I. The mean excess found in the samples from the Bolls-off group is expressed as a per cent. of the mean weight for the Bolls-on group.

TABLE I.

Percentage Increase of Bolls-off Group over Bolls-on Group in Weight per Sample.¹

	Water.	Dry Weight.	Total N.	Dry Weight - 5.7 N.	Polysaccharides.	Total Sugars.	Protein N.	Crystalloid N.
<i>Leaves.</i>								
Upper region	6.49	9.53	10.38	9.31	24.7	17.8	8.97	23.8
Lower region	3.10	4.92	12.62	2.86	12.35	15.4	12.92	10.3
Significant (P = 0.05)	2.96	5.64	7.69	5.51	9.16	13.9	7.75	10.2
Difference (P = 0.10)		4.47		4.38				
<i>Bark.</i>								
Upper region	13.90	20.10	39.3	18.65	54.1	21.7	37.2	46.7
Lower region	2.93	8.28	25.0	6.85	23.1	8.7	13.3	67.0
Significant (P = 0.05)	7.25	6.55	10.8	7.50	14.25	16.9	11.10	18.0
Difference (P = 0.10)	5.86			5.95		13.4		
<i>Wood.</i>								
Upper region	20.5	7.83	28.8	6.68	10.4	19.32	24.5	36.8
Lower region	6.25	1.58	20.4	0.86	8.4	9.35	16.5	27.2
Significant (P = 0.05)	8.25	6.40	9.5	7.30	11.35	15.7	10.75	9.05
Difference (P = 0.10)	6.65	5.15		5.89	9.15	12.45		

It will be seen that both carbohydrates and nitrogen have accumulated in the tissues of the Bolls-off group. Taking dry weight, less 5.7 times the weight of nitrogen, as an estimate of weight due to non-nitrogenous

¹ Statistically significant increases (P < 0.05) in heavy type, partially significant increases (P < 0.10) in ordinary type, all others in italic type.

compounds, mainly carbohydrates, we note that whereas the nitrogen response is everywhere significant the carbohydrate response is only significant in the Upper region of the plant. Also, the nitrogen response is very much greater than the carbohydrate response, except in the case of the leaves of the Upper region. These results confirm Murneek's findings that the developing fruits cause a bigger drain on the nitrogen than on the carbohydrate supplies of the plant. In the absence, however, of any exact knowledge as to the relative effects of nitrogen shortage and carbohydrate shortage on the activity of vegetative meristems, we cannot decide whether the retarding influence of the boll on vegetative growth is due mainly to its demand for nitrogen or to its demand for carbohydrates, or, for that matter, to its demand for some other substances such as phosphorus.

It will be noticed from Table I that the carbohydrate reserves (polysaccharides) have increased very much more than the dry weight, and in leaf and bark show an increase equal to or slightly greater than that of total nitrogen. The content of sugars also has increased to a greater extent than the increase in dry weight. Except in the wood, however, the increase in sugars is less than the increase in polysaccharides.

Comparing protein and crystalloid N it will be seen that, except in the leaves of the Lower region, the crystalloid N response is very much greater than that of protein. Also the response of crystalloid N is, especially in bark and wood, much greater than the response of total sugars.

Looking at the data from the point of view of transport of material, it will be seen that the removal of a 'sink' has increased the concentration of carbohydrates and nitrogen not only in the bark but also in the leaf and in the wood. In this respect the results are parallel to those obtained on ringing the stem, and confirm the conception of a gradient basis for transport of nitrogen and carbohydrates. It may be noted that our results differ from those of Murneek (12) in that we have found an increase in both carbohydrates and nitrogen on removal of the bolls, whereas he reports a decrease in carbohydrate and an increase in nitrogen on removal of the fruits of the tomato.

(b) *Sap concentrations.* The average concentrations found in the sap of the Bolls-on group are shown in Table II, and also the average increase or decrease in concentration in the Bolls-off group.

As already noted (Table I) the amounts of water present in the samples from the Bolls-off group are in all cases greater than those in the samples from the Bolls-on group. Hence an increase in concentration of any substance involves a somewhat greater increase in the actual amount of that substance.

In the *leaves* it is interesting to note that the increase in total sugars is mainly due to sucrose, reducing sugars showing very little change. If, as we have suggested earlier (11), reducing sugars form the head for export of

TABLE II.

Concentrations in Leaf, Bark, and Wood.

(Grm. Sugars or Mg. N per 100 grm. water.)

<i>Leaf.</i>					
	Bolls-on.		Increase or Decrease Bolls-off — Bolls-on.		Significant Difference. ($P=0.05$)
	Upper Region.	Lower Region.	Upper Region.	Lower Region.	
Total Sugars .	2.127	1.687	+ 0.247	+ 0.194	0.189
Sucrose .	0.324	0.231	+ 0.209	+ 0.164	0.086
Reducing Sugars .	1.803	1.456	+ 0.038	+ 0.030	0.344
Total N .	1510.6	1218.7	+ 56.0	+ 114.0	23.7
Protein N .	1361.2	1067.9	+ 31.5	+ 103.1	25.0
Total Cryst N .	149.4	150.8	+ 24.5	+ 10.9	4.82
Asparagine N .	11.71	10.96	+ 5.58	+ 1.72	9.64
Amino-acid N .	20.39	20.00	- 1.07	+ 2.68	4.16
Residual N .	101.60	111.86	+ 19.3	+ 3.27	13.6
Nitrate N .	12.60	5.73	- 0.35	+ 0.74	7.75
Ammonia N .	3.12	2.26	+ 1.02	+ 2.44	2.31
<i>Bark.</i>					
Total Sugars .	5.738	5.502	+ 0.406	+ 0.305	0.426
Sucrose .	2.732	1.623	+ 0.474	+ 0.799	1.396
Reducing Sugars .	3.006	3.879	- 0.068	- 0.494	1.080
Total N .	367.5	411.7	+ 82.7	+ 87.9	28.0
Protein N .	290.3	321.7	+ 60.2	+ 32.1	18.1
Total Cryst N .	77.16	89.98	+ 22.47	+ 55.77	17.9
Asparagine N .	25.09	40.86	+ 21.28	+ 47.45	6.91
Amino-acid N .	5.62	9.97	+ 0.84	- 1.02	1.54
Residual N .	31.91	30.63	+ 6.74	+ 6.58	14.5
Nitrate N .	13.63	7.18	- 6.41	- 0.61	5.51
Ammonia N .	0.91	1.34	+ 0.02	+ 3.37	2.90
<i>Wood.</i>					
Total Sugars .	1.987	1.809	+ 0.133	+ 0.052	0.176
Sucrose .	0.988	0.980	+ 0.139	+ 0.070	0.090
Reducing Sugars .	0.999	0.829	- 0.006	- 0.018	0.305
Total N .	400.2	375.2	+ 60.6	+ 49.7	28.4
Protein N .	260.4	239.4	+ 29.3	+ 22.9	28.7
Total Cryst N .	139.8	135.3	+ 31.3	+ 26.8	7.5
Asparagine N .	60.4	46.63	+ 26.7	+ 12.51	19.8
Amino-acid N .	12.31	10.06	- 1.29	+ 7.38	5.97
Residual N .	35.43	50.92	+ 13.79	+ 7.91	12.84
Nitrate N .	29.95	26.54	- 6.99	- 1.32	3.01
Ammonia N .	1.71	1.15	- 0.90	+ 0.29	1.66

Note.—The standard deviation, due to sampling, of the difference between the Bolls-on and Bolls-off groups has been calculated from the set of six differences, three for the Upper region and three for the Lower region, for each tissue. In the case of the leaves the differences in favour of the Bolls-off group tend to increase during the day. This drift has been allowed for in calculating the standard deviations. In view of the small number of degrees of freedom available for calculating the standard deviations the statistical data are put in the form of the Significant Difference for a probability of 5 per cent. This gives the appropriate weight to the standard deviations. Statistically significant increases or decreases are in heavy type, partially significant ones in ordinary type, others in italic type.

carbohydrate from the leaf, then this increase in sucrose (like the parallel increase in polysaccharides) represents temporary storage (3). In both cases the difference in favour of the Bolls-off group increased considerably during the day. The increase in protein N is greater than that in crystalloid N, as might be expected from the far higher concentration of protein N. The crystalloid increase is mostly due to residual N, but in part also to asparagine. This suggests¹ that residual N, which constitutes more than half the crystalloid N, may be the head for export of organic nitrogen from the leaf.

In the *bark* the increase in total sugars is entirely due to sucrose; but the sampling variation is abnormally large, and though this change is in the direction expected from earlier work, the result is not statistically significant. (The wood of the Upper region shows a significant response in sucrose.) The increase in total N is mainly protein N in the Upper region, and mainly crystalloid N in the Lower region. In both regions the change in crystalloid N is almost entirely a change in asparagine. Residual N also shows a small increase, but this is not statistically significant. It is interesting to note that nitrate N decreases, notably in the Upper region, where the decrease is quite significant.

In ringing experiments previously reported (8) we found that accumulation of nitrogen in the bark above a ring might be almost wholly protein N or almost wholly crystalloid N; and that where crystalloid N showed a response it was mainly an accumulation of asparagine. In the present experiment both protein and crystalloid N definitely respond, and, as before, the crystalloid N response is mainly asparagine.

In the *wood* the changes are, on the whole, similar to those in the bark, but here, in both Upper and Lower regions, the greater part of the response of total N is due to crystalloid N. As in the bark, the crystalloid N change is due mainly to an increase in asparagine. It is interesting to note that nitrate N shows a definite drop in the Upper region of the wood, just as in the bark.

To sum up the response shown by the different nitrogen compounds: the protein response is considerable in the leaves of the Lower region and the bark of the Upper; residual N shows a marked response in the leaves and wood of the Upper region, while asparagine responds markedly in the stem tissues and to a small extent in the leaf. The other compounds show only small changes.

The results are evidently in harmony with the view that residual N forms the head for nitrogen transport out of the leaf, and also that it is the form in which nitrogen escapes from the sieve-tubes into the wood. The

¹ This suggestion is supported by some preliminary observations on diurnal changes in the crystalloid fractions in the leaf. We have found that nearly the whole of the increase in crystalloid N during the day is due to residual N.

impossibility, however, of distinguishing between translocatory and storage compounds renders any conclusions somewhat doubtful. The results are essentially similar to those of ringing (cf. 8).

The *hydrogen-ion concentrations* are shown in Table III.

In all tissues except the bark of the Upper region the Bolls-off group shows a decrease in hydrogen-ion concentration (increase in pH); the change is, in all cases, small and only of about the same order as the standard deviation due to sampling. Any real change in pH as a result of removing the bolls is therefore doubtful. It will be noted that the bark sap is consistently more acid than the wood sap.

TABLE III.

Hydrogen-ion Concentration (pH).

	Bolls-on		Bolls-off		Standard Deviation of Difference.
	Upper.	Lower.	Upper.	Lower.	
Leaves	5.633	5.590	5.637	5.683	0.094
Bark	5.720	5.737	5.660	5.917	0.110
Wood	6.020	5.913	6.060	6.003	0.039

SECTION 3. THE UPTAKE OF NITROGEN AND OF CARBOHYDRATES BY THE YOUNG BOLL. THE EFFECT OF FERTILIZATION. (EXPERIMENT 15.)

When fertilization of the cotton boll is prevented the boll continues to grow for a few days (10) before being shed. The period during which growth continues varies with the conditions of nutrition; under good conditions (e.g. if there are very few other bolls on the plant) abscission may not take place for some ten or more days. It is clear that on fertilization some change takes place enabling the boll to maintain a rapid uptake of food material. It seemed, therefore, that a study of the uptake of nitrogen and carbohydrate, and of the changes in sap concentration, during the first few days of growth of fertilized and unfertilized bolls might throw some light on the means whereby this rapid uptake of nitrogen and carbohydrate by the boll is maintained. In addition, it was hoped that information might be obtained as to the changes leading to abscission of the boll.

Before presenting the results of this study it may be an advantage to consider in what way changes in sap concentration in a growing organ are to be interpreted. Let us assume that we have two organs having anatomically similar relations to the plant from which their supplies of food material are derived. One commences to grow, or increases its rate of growth; the other does not. The increased rate of growth may be due, either to an increased *rate of utilization* of the mobile compounds (i.e. the compounds

that enter the organ from the sieve-tube), or to an increased *ease of entry* of those mobile compounds, or to both causes in conjunction. In the first case, the rate of utilization (transformation into growing tissue) of the mobile compounds present in the organ increases; the concentration of these compounds in the organ decreases; the gradient into the organ steepens, and in consequence the rate of uptake increases. In the second case, the rate of movement for unit gradient of entry of mobile compounds increases, the concentration of mobile compounds in the organ increases, and transformation (i. e. growth) becomes more rapid. Thus, in the first case, increased rate of utilization, there is a drop in concentration, and in the second case, increased ease of entry, there is an increase in concentration. If both are in operation, there may, of course, be no change in concentration.

This increase in ease of entry may be due to an increased permeability of the tissues of the growing organ which lie between the conducting cells and the meristems, or the permeability change might be restricted to the membranes of the conducting cells themselves. One further possibility (cf. Curtis, 1), of considerable interest, may be noted. The high rate of spread of food materials about the plant seems to be due to some mechanism in the sieve-tubes (cf. 11), which enormously accelerates the process of mixing. This mechanism *may* be protoplasmic streaming, though there is no conclusive evidence of streaming in the mature sieve-tube. Until we have such evidence it seems preferable to speak merely of an acceleration mechanism. Now the marked effect of temperature (cf. 2, 4) on rate of translocation must be very largely due to an effect on this acceleration mechanism, for the temperature coefficient is apparently far greater than that for physical diffusion. If temperature can so markedly affect acceleration it seems reasonable to suppose that internal factors also may have a profound influence. Admitting such an influence of internal factors, it is clear that sieve-tubes in different regions of the plant may exhibit quite different accelerations, and that the acceleration in any one region may vary with the metabolic state of the plant or of that region. The distribution of food materials among the organs of the plant might thus be determined, in part, by the particular rates of acceleration obtaining in the sieve-tubes leading from the main system to each organ. For example, the increased rate of growth of the fertilized, as compared with the unfertilized, boll might be due to an increased acceleration in the sieve-tubes of the pedicel of the boll. Much further experimental work will be required before this possibility can be tested. For the purpose of the present discussion it is sufficient to note that the three possible changes which we have indicated—(1) increased permeability of the boll tissues, (2) increased permeability of the membranes bounding the sieve-tubes of the boll, (3) increased acceleration in the sieve-tubes leading to the boll—will have similar effects on the average concentrations of mobile compounds in the sap of the boll tissues. We shall, there-

fore, include all three possibilities under the general head, increased ease of entry of mobile compounds.

We distinguish, accordingly, two types of changes as likely to lead to an initiation or resumption of growth, or an increased rate of growth—(1) increased rate of utilization, and (2) increased ease of entry of mobile compounds. In the first case the concentration of mobile compounds in the growing tissues will fall; in the second case, the concentration will rise. If, on other grounds, we have already identified the mobile compounds, then a study of the concentration changes accompanying the initiation of growth, or increase in rate of growth, of an organ will enable us to say whether the increased growth is due to increased rate of utilization or increased ease of entry. If we do not already know, but wish to identify, the mobile compounds, then clearly we must have some information, or make some assumptions, as to the likelihood or otherwise of increased rate of utilization as against increased ease of entry.

This fact, that identification of the mobile compounds is dependent on some knowledge of, or some assumptions as to, the type of change responsible for the increased growth has not, so far as we are aware, been previously emphasized.

In the case of the cotton boll we have suggested in our earlier paper that carbohydrates may enter it as sucrose; the mobile nitrogen compound is, as yet, unidentified, though there are grounds for thinking that nitrogen may leave the sieve-tube as residual N. In interpreting the changes in sap concentration in fertilized and unfertilized bolls we shall, therefore, examine the conclusions that would follow if (a) the effect of fertilization is mainly an increased rate of utilization or if (b) the effect is mainly on the ease of entry of mobile compounds.

(1) Procedure.

In order to simplify the problem it was decided to make observations, not on the boll as a whole, but separately, on (1) the *ovules*, (2) the remaining tissues of the boll, which for convenience we term *carpels*. In order to obtain sufficient sap a very large number of bolls was required. It was not possible, therefore, to make more than one collection of bolls from the flowers that opened on any one day. To meet this difficulty the collection of bolls of different ages was arranged as shown in the table below.

It will be seen that there were two series: series A with bolls 1, 3, 5 and 7 days old, and series B with bolls 0, 2, 4 and 6 days old.

The flowers were tagged with coloured wool on the evening before they opened. In approximately half the total number fertilization was prevented by removal of the stigmas at dawn, before the corolla opened.

The normal and unfertilized bolls were subsequently collected in samples of about 400 bolls for each. Four such samples of each were taken

during the day—two in the morning and two in the afternoon. Ovules and carpels were separated and weighed; and samples were taken in the usual way for determination of dry weight and for expression of sap.

Time Table for Collection of Bolls.

	Flowers opened on	Bolls collected on	Age in days at time of collection.
<i>Series A.</i>			
1928	March 22	March 29	7
	" 23	" 28	5
	" 24	" 27	3
	" 25	" 26	1
<i>Series B.</i>			
	March 30	April 5	6
	" 31	" 4	4
	April 1	" 3	2
	" 2	" 2	0

The determinations on the sap were made on the bulked material from the four samples collected on each day, and comprised crystalloid N, asparagine N (including ammonia), amino-acid N, sucrose, reducing sugars and hydrogen-ion concentration. Total N was determined on the dried material.

In addition, samples of bark were collected each day from the fruiting branches bearing the bolls, and the usual determinations were made.

(2) *Results.*

(a) *Uptake of dry material, nitrogen, and water.* Before considering the curves obtained for increase in dry weight and in nitrogen, it should be noted that bolls on the point of undergoing abscission were rejected from both groups. These bolls are easily recognized by their lack of turgor. The percentage of these 'soft' bolls in the Normal group was throughout small, rising only from 0.6 per cent. on day 2 to 3.0 per cent. on day 7. In the Unfertilized group the percentages from day 2 to day 7 were 0.5, 1.0, 2.5, 6.0, 13.4, 24.9. At this rate of increase all the bolls in the group would have been shed by about the tenth day. The 'soft' bolls were rejected because it did not seem desirable to include in any collection bolls that *might* not be represented in a collection made two days later. If no selection had been made, somewhat smaller weights would have been obtained for the Unfertilized group, since the average weight of the 'soft' bolls was about 70 per cent. of the average weight of the other bolls. Inclusion of the 'soft' bolls would have given on the seventh day weights about 1 per cent. lower for the Normal group and about 7.5 per cent. lower for the Unfertilized group; on other days the effect would have been negligible.

With the exception of these 'soft' bolls, which formed a quite distinct

class in each group, the samples of unfertilized bolls were as uniform as those of the normal bolls, both sets showing unimodal size distributions. If, in spite of the removal of the stigma, fertilization had actually occurred

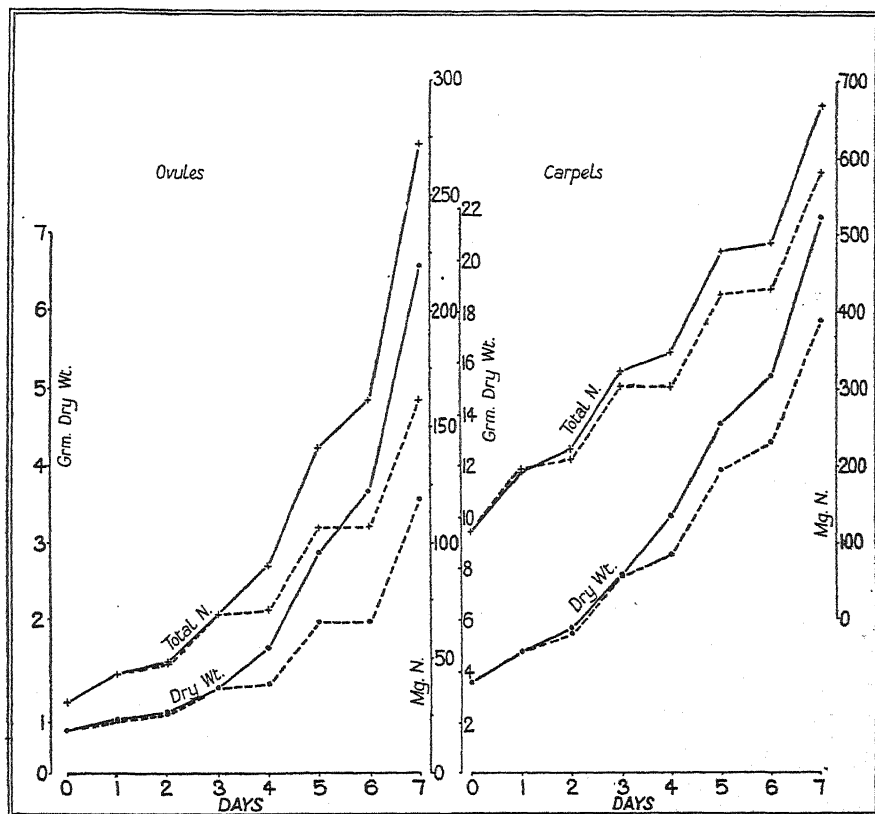


FIG. 1. Grm. dry weight and mg. nitrogen per 100 bolls in ovules and carpels. Normal group continuous, and Unfertilized group broken line.

in an appreciable number of the bolls of the Unfertilized group, the samples of this group should have shown, from the fourth day onwards, a marked bimodality. It seems, therefore, reasonable to assume that the figures obtained for the Unfertilized group fairly represent growth that has taken place in the absence of fertilization.

The weights of dry material and of total nitrogen in ovules and carpels, per 100 bolls, are shown graphically in Fig. 1. The Normal group is represented by a continuous line, the Unfertilized by a broken line. Series A (days 1, 3, 5, 7) and series B (days 0, 2, 4, 6) have been combined in drawing the figure. It will be seen that, if a smooth curve were drawn through the values for each group, the values for series A would lie somewhat above, and those for series B somewhat below the smooth curve. Growth is clearly proceeding somewhat more rapidly in series A than in series B.

For the first three days after flowering there is no appreciable difference between Normal and Unfertilized groups. By the fourth day the ovules and carpels of the Unfertilized group show a decided lag, both in dry weight and in nitrogen, and this lag becomes more marked as time goes on. But, although the unfertilized ovules and carpels do exhibit a marked depression, as compared with the normal, growth does continue at a considerable rate. This long-continued growth, especially that of the ovule, is surprising, and it is remarkable also that, for the first three days after flowering, no difference in growth between Normal and Unfertilized groups should have been apparent.

Microscopic examination of fixed material showed that in the Normal group the endosperm nucleus had begun to divide by the second day, but that the first division of the ovum did not take place until the fourth day. By this time a considerable number of endosperm nuclei had been formed. In the Unfertilized group there were a few cases where fertilization had undoubtedly taken place, for the remains of the pollen tube could be seen. In these cases development was exactly as in the normal ovule. Except in these few cases, however, the ovules of the Unfertilized group showed no division, either of the endosperm nucleus or of the ovum. It is interesting to note that the day on which the first division of the ovum occurred is the first day on which the fertilized ovules and carpels show any increase over the unfertilized. The effect of fertilization on growth must, accordingly, be due to some change which takes place prior to the first division of the ovum. The earlier division of the endosperm nucleus may have some significance in this connexion.

As Fig. 1 shows, fertilization causes an increased uptake both of dry material and of nitrogen. In both cases the effect is first apparent on the fourth day. In order to compare the uptake of nitrogen and carbohydrate in greater detail, we give in Table IV the actual weights of dry material, of total N, and also of water, per 100 bolls, on each day of the experiment. From these figures we have calculated, for each day from 1 to 7, the uptake since day 0, and have expressed the value for the Normal group as a percentage of the corresponding value for the Unfertilized group. As an approximate measure of carbohydrate we take the dry weight minus 5.7 times the weight of nitrogen. The results are given in Table V.

Taking the ovules first (Table V*a*), up to and including day 3, the Normal group shows no appreciable increase in uptake. On day 4 there is a sudden increase in uptake of all materials, and this increase becomes greater as time goes on. Carbohydrate uptake increases most, water uptake a little less, and nitrogen uptake very much less. A further point of interest is that the divergence between Fertilized and Unfertilized groups is greater for series B (days 0, 2, 4, 6), which, as we noted earlier, exhibited a slower rate of growth than series A (days 1, 3, 5, 7).

The behaviour of the carpel is, in general, similar to that of the ovules, though the percentage increases, as might be expected, are very much smaller. There is the same rather sudden increase in uptake on day 4, but

TABLE IV.

Grm. Dry Weight, Nitrogen, and Water per 100 Bolls

Day.	<i>Normal Group.</i>					
	Ovules.			Carpels.		
	Water.	Dry Weight.	Nitrogen.	Water.	Dry Weight.	Nitrogen.
0	1.93	0.559	0.0318	10.36	3.57	0.115
2	3.11	0.802	0.0482	20.50	5.64	0.221
4	7.80	1.627	0.0900	38.22	10.03	0.347
6	21.78	3.667	0.1615	56.28	15.50	0.488
1	2.56	0.702	0.0430	16.33	4.74	0.193
3	4.76	1.123	0.0686	30.04	7.76	0.322
5	16.76	2.866	0.1414	51.47	13.65	0.479
7	43.60	6.635	0.2721	82.10	21.70	0.685
<i>Unfertilized Group.</i>						
0	1.93	0.559	0.0318	10.36	3.57	0.115
2	2.93	0.784	0.0477	20.01	5.43	0.208
4	5.46	1.176	0.0707	31.44	8.53	0.304
6	11.27	1.985	0.1070	45.30	12.93	0.430
1	2.54	0.695	0.0432	16.49	4.78	0.196
3	4.71	1.124	0.0687	29.59	7.71	0.308
5	11.04	1.977	0.1066	42.55	11.85	0.423
7	22.84	3.565	0.1622	61.85	17.68	0.582

there is not, as in the case of the ovule, a further increase on subsequent days. As in the ovule, nitrogen uptake exhibits the smallest increase, but the increase in water uptake, instead of being somewhat less than that in carbohydrate, is slightly greater.

From this point of view the effect of fertilization may be summed up as a marked increase in carbohydrate uptake, a slightly smaller (ovules) or slightly larger (carpels) increase in water uptake, and a much smaller increase in uptake of nitrogen. A possible interpretation of this result is that the primary effect is on carbohydrate uptake. With increased carbohydrate content there would be both greater hydration capacity and a more rapid utilization of mobile nitrogen compounds, the latter leading to a steepening of the gradient of entry of mobile nitrogen. In consequence, uptake of water and of nitrogen would increase, but to a less extent, perhaps, than uptake of carbohydrate. If this view is correct the effect of fertilization on

carbohydrate uptake would be due to increased ease of entry of mobile carbohydrates, coupled perhaps with increased rate of utilization, while the increased uptake of nitrogen would be due mainly to the second cause, increased rate of utilization.

TABLE V.

Uptake of Dry Weight, Nitrogen, and Water by Fertilized Bolls expressed as a Percentage of the Uptake by Unfertilized Bolls.¹

(a) *Ovules.*

Day.	Water.	Dry Weight.	'Carbohydrate' Dry Weight, less 5.7 N.	Nitrogen.
1	103.4	105.7	113.0	97.9
2	117.8	108.2	112.1	102.7
3	101.8	99.8	99.7	99.8
4	166.4	172.7	186.6	149.5
5	162.9	162.6	169.8	146.4
6	212.8	217.8	237.5	172.4
7	199.2	202.4	207.9	184.5

(b) *Carpels.*

1	97.4	97.1	97.7	96.2
2	105.0	111.2	110.5	113.3
3	102.4	101.4	99.3	107.3
4	132.1	130.4	132.3	122.7
5	127.7	122.0	122.7	118.3
6	131.6	127.6	129.7	118.3
7	138.9	128.5	130.0	122.0

It must be noted, however, that the greater apparent effect on carbohydrate uptake as compared with nitrogen uptake does not necessarily imply that the carbohydrate effect is primary and the nitrogen effect secondary. As the boll grows the ratios of carbohydrate, nitrogen and water in the material taken up are changing continuously. In Fig. 2 A are shown the ratios of nitrogen and of water to dry weight for the ovules and carpels of both groups from day 0 to day 7.

In both ovules and carpels the proportion of nitrogen rises on the first day and is then approximately constant up to the third day. Up to this point also there is little difference between Fertilized and Unfertilized groups. On day 4 there begins in both groups a marked drop in proportion of nitrogen, but the fall is much more rapid in the Fertilized group. Consequently from the fourth day onwards the proportion of nitrogen is much less in this group.

¹ For each day the increment in the Normal group since Day 0 is expressed as a percentage of the corresponding increment in the Unfertilized group.

The drift is, however, very similar in the two groups, and it appears as if the fertilized bolls were changing in the same way as the unfertilized but

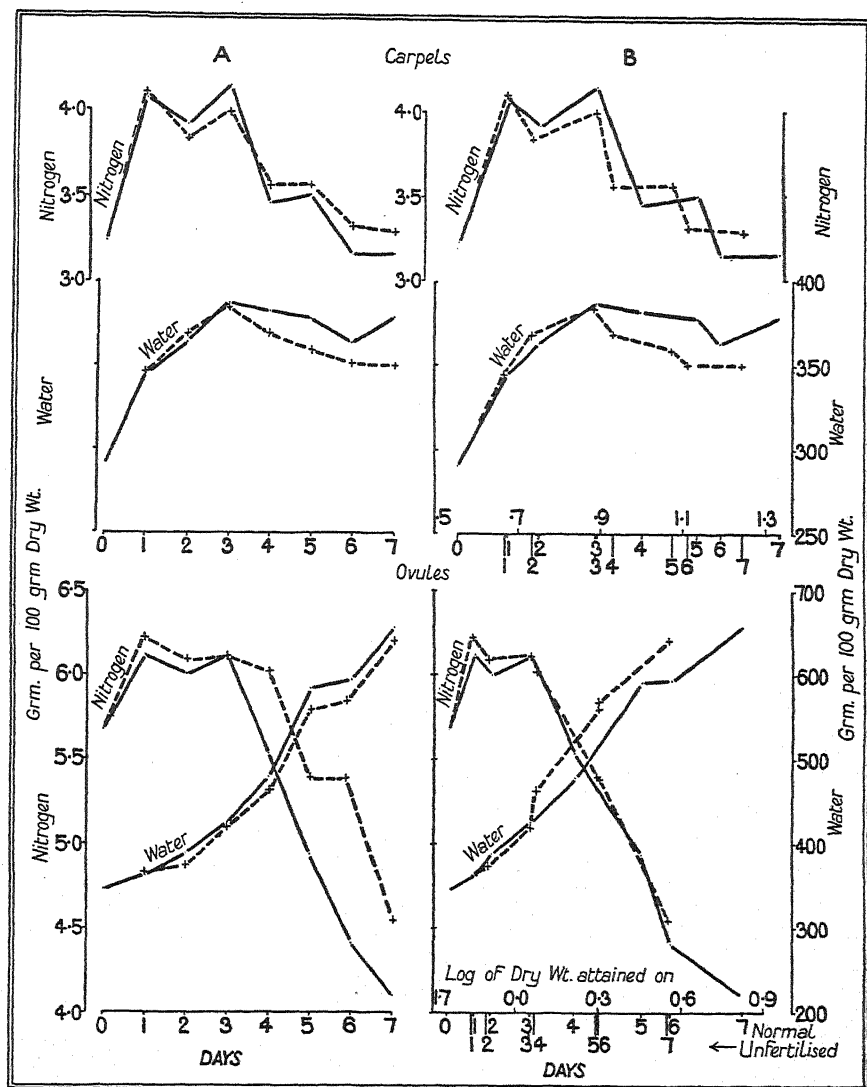


FIG. 2. Grm. water and nitrogen per 100 grm. dry weight plotted A (left) against days ; B (right) against logarithm of dry weight attained on days 0-7. Normal group (continuous line), and Unfertilized group (broken line). In the bottom right-hand graph for 1.7 on the base line read 1.7.

at a faster rate. The curves suggest that the fertilized bolls have a lower proportion of nitrogen on any day after the fourth because they have reached a more advanced stage in growth and differentiation. To test this possibility we may compare the two groups, not after equal intervals of

time, but after the same amount of dry material has been taken up. For this purpose the values have been plotted again in Fig. 2 B, not against time, but against the logarithm of the dry weight (logarithms being used so as to have approximately equal intervals between days). It will be seen that the nitrogen curves for the two groups are now indistinguishable.

Thus the proportion of nitrogen present is determined by the dry weight attained: equally, however, the dry weight attained may be regarded as determined by the total weight of nitrogen taken up. It is clear that the *course* of carbohydrate uptake and that of nitrogen uptake are equally accelerated by fertilization and, though the primary effect *may* be on carbohydrate uptake, we must have other evidence before asserting that this is so.

Fig. 2 shows, in addition to the nitrogen curves, curves for moisture content (weight of water per 100 grm. dry weight). Plotted on the ordinary time scale (Fig. 2 A), in both groups the ovules show a rapid rise in moisture content, but the rise is steeper in the Normal group, which is growing and differentiating at a faster rate. When, however, we compare moisture contents for equal dry weights attained (Fig. 2 B), the fertilized ovules have not more, but less, water. It would appear as if the slower growth of the unfertilized ovule allowed of more complete hydration of the material taken up.

In the carpels the situation is different. Moisture content increases in both groups up to day 3: after that day it remains fairly steady in the Normal group but falls somewhat in the Unfertilized. Plotted against dry weight attained the divergence is, if anything, more marked. The new tissue formed in the carpel as a result of fertilization is apparently more highly hydrated than the new tissue formed in the absence of fertilization.

These moisture changes are of interest in connexion with the suggestion that the shedding of bolls may be due to a reduction in moisture content. The behaviour of the carpels is in harmony with this suggestion, but the contrary behaviour of the ovules makes such an explanation of the shedding of unfertilized bolls improbable.

To sum up the growth data, the effect of fertilization appears rather suddenly on the fourth day after anthesis. From this day onwards the rate of uptake of carbohydrate, nitrogen and water is increased, the increase being much more marked in the ovules than in the carpels. No distinction can be drawn between the effect on carbohydrate uptake and the effect on nitrogen uptake. In the ovules hydration lags behind uptake of dry matter, but in the carpels the hydration capacity is somewhat increased by fertilization.

(b) *Sap concentrations.* Before considering the changes in sap concentration in the bolls, and the possible bearing of these changes on the uptake differences already noted, some comment may be made on the con-

centrations recorded for the bark of the fruiting branches. Samples of bark were collected along with the bolls, so that we have two series, A (days 1 to 7) and B (days 0 to 6). The average concentrations for each series are given in Table VI.

TABLE VI.
Concentrations in Bark of Fruiting Branches.
(Grm. carbohydrate or mg. N per 100 grm. water.)

	Series A.	Series B.
Total sugars . . .	3.38	3.17
Sucrose . . .	1.72	1.47
Reducing sugars . . .	1.66	1.70
Total N . . .	660	704
Protein N . . .	438	444
Crystalloid N . . .	222	260
Asparagine N . . .	95.2	109.1
Amino-acid N . . .	14.4	15.9
Residual N . . .	77.8	113.1
Ammonia N . . .	3.1	3.1
Nitrate N . . .	31.5	18.8

It will be remembered that growth (uptake of carbohydrate and of nitrogen) was more rapid in series A than in series B. It is interesting, therefore, to note that the bark of series A has a higher concentration of sucrose. The same is true for nitrate nitrogen (see p. 678). The other nitrogen fractions, however, have a lower concentration in series A. As they stand, the results suggest that the growth of the boll may be determined mainly by the availability of sucrose, fluctuations in nitrogen supply being relatively unimportant. The bark data refer, however, only to the latter half of the growth period of the bolls in each series, and the difference between A and B cannot be stressed. The data are chiefly of value in giving an approximate measure of the levels of concentration in the bark adjacent to the bolls. In comparing concentrations in bark and boll, it should be remembered that certain compounds are present in much higher concentrations in the sieve-tubes than in the bark as a whole. In the bark of the main axis, for example, we obtained (6) estimates of concentration in the sieve-tubes about three times as high as those in the bark as a whole, for the following compounds: sucrose, amino-acid N, residual N. Protein N, asparagine N, and nitrate N had about the same concentration as in the whole bark, while reducing sugars had a lower concentration. Naturally, we cannot apply these ratios to the present case; the disparity between bark concentration and sieve-tube concentration may here be greater or less than that quoted; but it seems reasonable to assume that it will be in the same direction.

The march of *sap concentrations in the ovules* is shown in Fig. 3, a broken line being used for the Unfertilized group and a continuous line for the Normal. The results for series B are on the left, and those for

series A on the right. The average concentration in the bark of the fruiting branches is shown by short horizontal lines on the left of the graphs.

Taking first the results for *sugars*, on day 0 the concentrations of sucrose and of reducing sugars are only slightly below the average concentrations in the bark. Sucrose then drops rapidly and remains low throughout: reducing sugars remain almost constant until the third day, after which a very rapid increase begins. This rise in concentration of reducing sugars is much steeper in the normal ovules so that, on any day after day 3, the normal ovules have a much higher concentration. In sucrose concentration, however, there is no appreciable difference between normal and unfertilized ovules. The concentrations are, of course, very small and only a relatively large difference would be detected with certainty.

As a result of our earlier work on carbohydrate transport (11) we suggested that carbohydrates enter the boll as sucrose, the rapid uptake by the boll being due to the very steep sucrose gradient maintained. The general course of events in the ovule supports this view. The sucrose concentration is well below that in the bark and the gradients from the sieve-tube will be much steeper. The reducing sugar concentration, on the other hand, very soon rises above that in the bark and is probably, even from the start (day 0), higher than that in the sieve-tubes. Now if carbohydrates do enter as sucrose, it is clear that the ease of entry of sucrose must be much greater in the fertilized ovule, for the gradient remains approximately the same in the two groups, though uptake increases considerably. At the same time the rate of utilization (transformation) of sucrose must have increased, for although carbohydrates enter more rapidly the concentration of sucrose remains the same.

Thus, if carbohydrates enter as sucrose, the effect of fertilization is both increased ease of entry and increased rate of utilization. If, on the other hand, reducing sugars are the mobile compounds, the effect of fertilization is one mainly on ease of entry. Ease of entry cannot be exactly measured, since we do not know the effective gradients of entry. But we can make an estimate of the rate of utilization in the following way.

If the total uptake of carbohydrate, or of nitrogen, during unit interval of time is T and the hypothetical mobile compound, M , has also increased by the amount m , then the total amount of carbohydrate or nitrogen utilized or transformed is $T-m$. The rate of utilization is then the amount utilized, $T-m$, divided by the mean amount of M present during that time interval. In calculating *Utilization indices* from the present data we have taken series A and series B separately, and have used a unit interval of two days. Each compound studied is in turn assumed to be the mobile compound, for nitrogen or for carbohydrate uptake, and utilization indices are calculated. The indices are shown in Fig. 4 plotted at the midpoints of the time intervals to which they refer. The vertical scales used differ for different compounds,

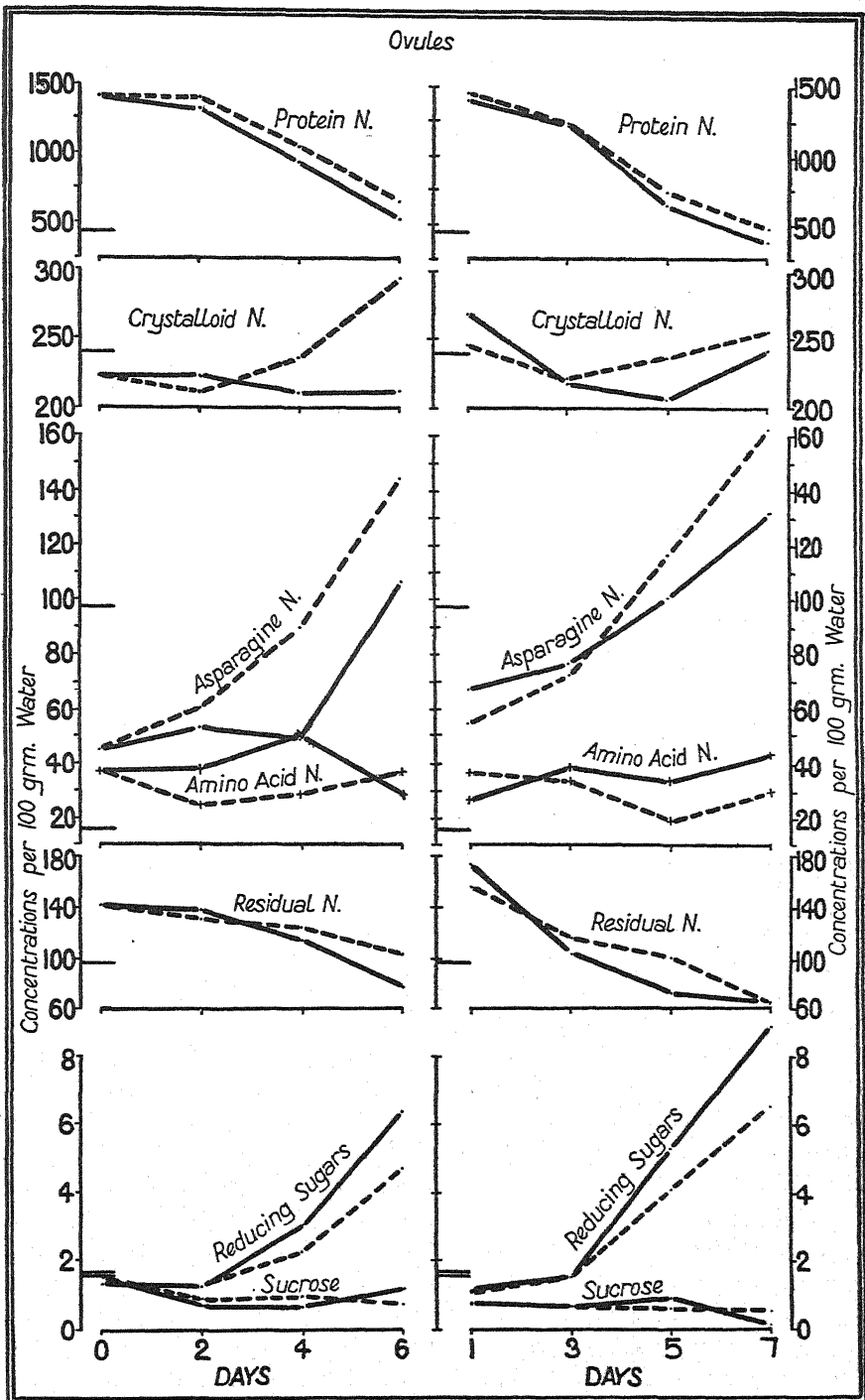


FIG. 3. Grm. sugar and mg. nitrogen per 100 gm. water in ovules of Normal group (continuous line), and Unfertilized group (broken line).

and are so arranged that the mean values for the Normal group (shown by the horizontal lines on the left of the graphs) have the same linear dimensions in all graphs. The curves for the Unfertilized group are, as usual, distinguished by the broken lines.

It will be seen that the indices for sucrose are much higher in the Normal group, but that those for reducing sugars are almost exactly the same for the two groups. The rapid fall in rate of utilization of reducing sugars is very interesting. By the seventh day reducing sugars constitute more than half the dry weight of the ovule. The rate of transformation of sucrose, on the other hand, is increasing. On the whole it seems probable that sucrose is the mobile compound and, consequently, that the effect of fertilization is both an increased ease of entry and an increased rate of utilization.

Coming to the *nitrogen compounds* in the ovule (Fig. 3) it will be seen that, as growth proceeds, the concentrations of protein N, and also of residual N, fall; total crystalloid N and amino-acid N show no definite trend, but asparagine N increases.

Comparing the concentrations in the ovules with those in the bark, protein N is initially much higher in the ovule and only reaches the bark level by about the seventh day. Total crystalloid N is somewhat lower than in the bark and much lower, therefore, than in the sieve-tubes. Asparagine begins lower, but after the fifth day is higher than in the bark and probably, therefore, higher than in the sieve-tubes. Amino-acid N is much higher than in the bark, though it may still be less than in the sieve-tube. Residual N, again, is initially higher in the ovule, but it falls during development, and may at all points be below the concentrations in the sieve-tubes. We cannot, of course, tell how much of the asparagine N, residual N, etc., that we determine in the sap of the bark or of the ovule is storage material and how much is really free to move. The results do, however, suggest in the first place that nitrogen enters as crystalloid N, and that, at any rate from the third day onwards, residual N rather than asparagine N is the important compound. Whether amino-acids play any part or not it is difficult to judge. It should be noted that ammonia N and nitrate N were not determined in the ovule except on days 6 and 7. The asparagine N in the ovule therefore includes ammonia N, and residual N includes nitrate. The concentrations of ammonia and nitrate N on the sixth and seventh days were low (ammonia N 7.3 mg. and nitrate N 1.3 mg.) so that the results are probably not appreciably affected. From the low concentration of nitrate N in the ovule it is possible that some nitrogen enters as nitrate, for the mean concentration in the bark is about 20 mg. (per 100 grm. water), and the concentration in the sieve-tube is about the same as that in the whole bark. This could hardly, however, account for more than a small part of the total uptake.

Comparing concentrations in the two groups it will be seen that, from

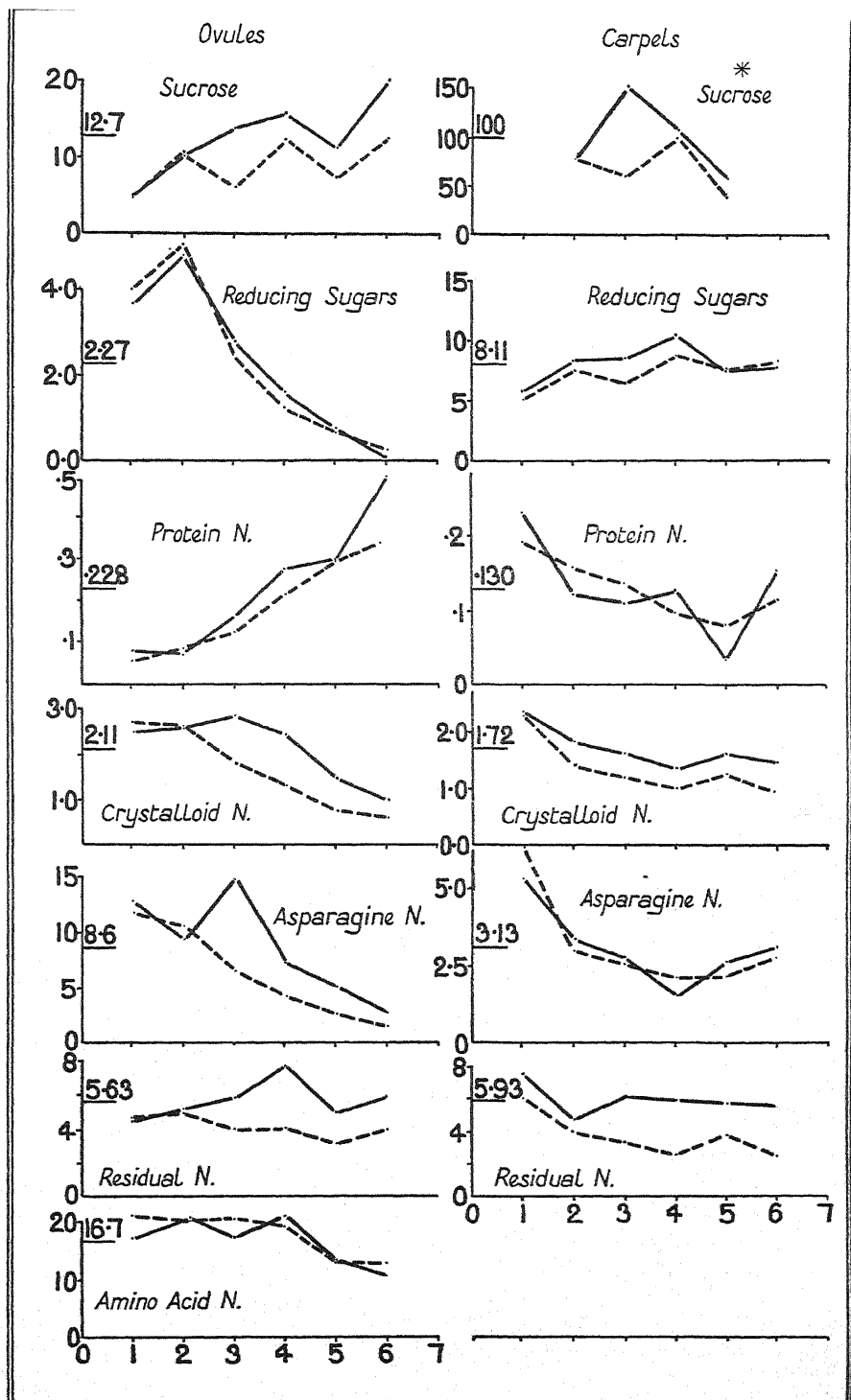


FIG. 4. Indices of utilization in Normal group (continuous line), and Unfertilized group (broken line). Four-day intervals have been used for the sucrose calculations in the carpels. In all

the fourth day onwards, the unfertilized ovules have a higher concentration in all nitrogen fractions except amino-acid. In the case of protein N this may be interpreted as a slower downward drift of concentration, due to the slower rate of differentiation and growth, for when the concentrations are plotted against dry weights attained (Fig. 5), the Unfertilized group has actually a lower concentration. Crystalloid N, however, shows no definite drift as the ovule grows, and the increase in the unfertilized ovule becomes even more striking when plotted against the dry weight scale. It will be noted that this divergence in concentration begins at the same time as the divergence in nitrogen uptake, and is much more marked for series B (days 0-6), which showed also the greater divergence in nitrogen uptake. The results strongly suggest that the greater uptake of nitrogen by the fertilized boll is due to a greater rate of utilization of crystalloid N, producing a lower concentration and a steeper gradient into the ovule.

As to the particular crystalloid fraction responsible, the evidence is not quite as clear. Amino-acid N may be excluded, for it has a higher concentration in the fertilized ovule and would seem, therefore, to be one of the products of transformation rather than the mobile compound. The greater part of the crystalloid N effect is due to asparagine, but residual N also shows a definite response. Considering only the divergence between normal and unfertilized ovules, either asparagine or residual N, or both, might be the mobile compound. As against asparagine we have, however, (1) the high concentration in the ovule, as compared with the bark, after day 4, and (2) the fact that in series A, which is taking up nitrogen more rapidly, the concentration is higher, and the gradient of entry consequently smaller, than in series B. Residual N, on the other hand, has a low concentration after day 4 and the concentration is somewhat lower also in series A than in series B. This argument in favour of residual N is diminished somewhat by the fact that when the concentration drifts are plotted against dry weight attained (Fig. 5) the divergence in residual N between the two groups is much less marked, while that in asparagine is emphasized. A possible interpretation of the results is as follows. Nitrogen enters as residual N and is converted to asparagine N and amino-acid N and protein N. Fertilization increases the velocity of conversion of residual N and of asparagine N into amino-acid and protein N, but has a smaller effect on the velocity of the change from residual N to asparagine N. The result is an increase¹ in concentration of protein N and amino-acid N, a large decrease in asparagine N and a smaller decrease in residual N. Whether the decrease in residual N concentration is sufficient of itself to account for the increased uptake cannot be said: possibly fertilization also increases ease of entry of residual N.

The utilization indices, as will be seen from Fig. 4, indicate very little

¹ These increases or decreases are, of course, relative to the unfertilized ovules.

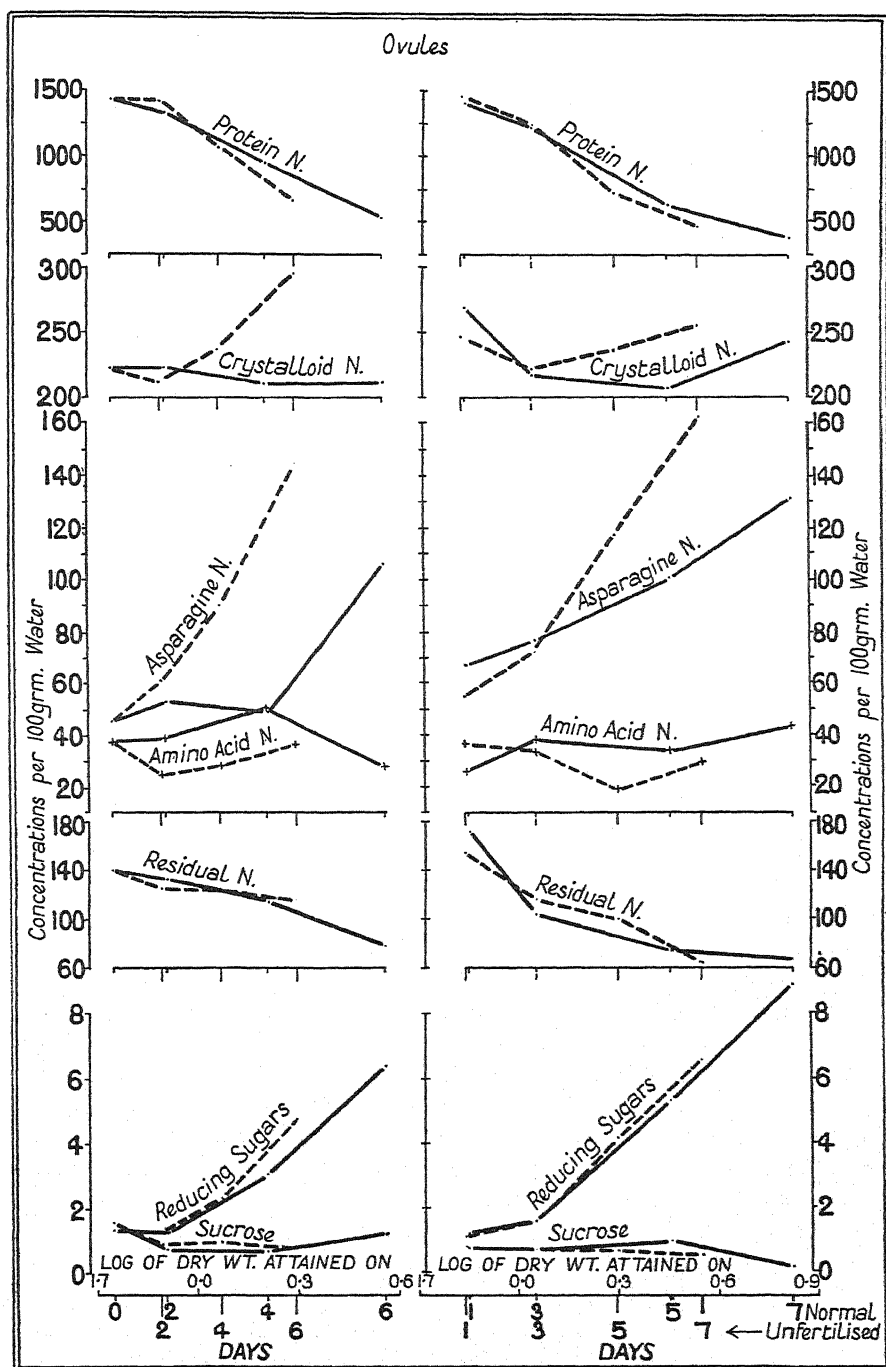


FIG. 5. Grm. sugar and mg. nitrogen per 100 gm. water in ovule plotted against logarithm of dry weight attained on days 0-7 in Normal group (continuous line), and Unfertilised group (broken line).

effect of fertilization on protein and amino-acid N, but a marked effect on residual N and asparagine N. It is interesting to note also that the indices for amino-acid N and residual N change very little during development.

The march of *sap concentrations in the carpels* is shown in Fig. 6 and utilization indices for the different compounds are given in Fig. 4. Owing to the very slight drift of concentration with growth it is not necessary to show the concentrations on the dry weight scale as well as the time scale.

Taking sugars first, the sucrose curves are like those in the ovule but at a much lower level of concentration. There is very little difference in concentration between Normal and Unfertilized groups. Reducing sugars do not show the marked rise that occurred in the ovule, and again there is little difference between the two groups, though after day 4 the Normal appears definitely higher. Increased carbohydrate uptake is clearly due to increased ease of entry, coupled with increased rate of utilization. Owing to the very low figures obtained for sucrose the utilization indices for this compound have been calculated by taking 4-day intervals 0-4, 2-6, 1-5, 3-7. Both reducing sugars and sucrose show increased indices on fertilization and on the data no satisfactory distinction can be drawn between them. The very steep gradient of entry for sucrose suggests, however, that carbohydrates enter as sucrose.

In the case of *nitrogen compounds* only protein N shows any marked drift with time. There is no clear difference between the two groups for this fraction. Crystalloid N, however, shows, as in the ovule, a higher concentration in the Unfertilized group after day 4. This divergence in crystalloid N is due almost entirely to residual N. Asparagine, which contributed most to the similar divergence in the ovule, is here not consistently different in the two groups. Amino-acid N also shows no consistent difference.¹ Similarly the indices of utilization in the two groups (Fig. 4) are not appreciably different for protein N, and asparagine N, but for residual N the Normal group shows a marked increase over the Unfertilized. It seems very probable, therefore, that the increased uptake of nitrogen by the fertilized carpel is due to increased rate of utilization of residual N, and that this is the form in which nitrogen enters. If, on the other hand, asparagine and amino-acids are the mobile compounds the effect of fertilization is mainly increased ease of entry.

It will be seen that, as we emphasized in the earlier discussion (pp. 665-7), we can form only contingent conclusions as to the mobile compounds and the type of change produced by fertilization. The recognition of this

¹ It will be noted that some negative values were obtained for amino-acid N. The significance of this has been discussed briefly in an earlier paper (6). We have since found that with the technique followed in clearing the sap for the determination of amino N, there is liable *in the carpel* to be incomplete recovery of amino N. This is due to the very viscous nature of the sap expressed from the carpel.

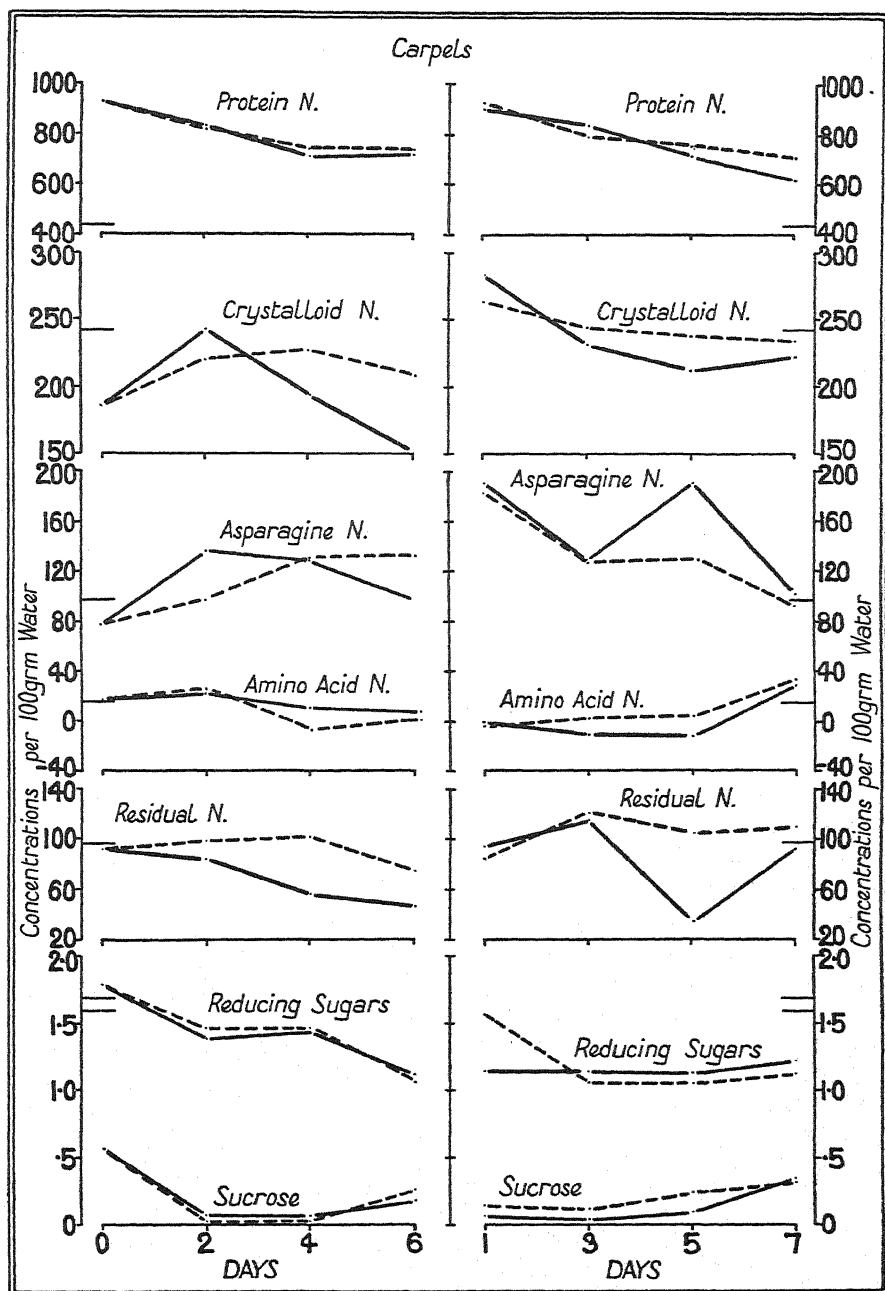


FIG. 6. Grm. sugar and mg. nitrogen per 100 grm. water in carpels of Normal group (continuous line), and Unfertilized group (broken line).

contingency is, however, in our view, fundamental to an understanding of the mechanism of uptake by an organ, and it has seemed desirable to exhibit the various related possibilities somewhat fully rather than to draw uncritical conclusions.

The case is, perhaps, most clear for carbohydrates. If these enter as sucrose *or* as reducing sugars, in either case fertilization must have increased the ease of entry, for the gradient of entry is no greater or is actually less in the fertilized boll. If, as seems probable on other grounds, the mobile compound is sucrose, rather than reducing sugars, we have in addition an increased rate of utilization. The drain in carbohydrates which the growing boll exercises would thus be due (1) to a high rate of utilization, maintaining a low concentration of sucrose in the boll, (2) to great ease of entry of sucrose into the boll. Whether, however, the ease of movement of sucrose into the boll is greater than towards a vegetative meristem we cannot say. Again, the gradient of sucrose into the carpel is much steeper than into the ovule, yet the relative growth rate of the ovule is far greater. It would be interesting to know whether this is due to a higher ratio of conducting to growing tissues, or whether sucrose escapes from the sieve-tubes more easily into the ovule tissues than into the carpel tissues.

The nitrogen problem is rather more complex. In the ovule, however, the higher utilization indices obtained in the fertilized ovules for all fractions except amino-acid N, indicate that increased uptake is probably due in part to an increased rate of utilization. With the exception of amino-acid N again the gradients of entry for all crystalloid fractions are steeper into the fertilized ovule, so that increased *ease* of entry of mobile nitrogen may not be involved. Of the nitrogen fractions in the carpel, only residual N shows a definitely increased utilization index and a steeper gradient of entry for the Normal group. The other fractions show little change, either in concentration or in utilization index.

It will be seen that increased utilization (due possibly to the higher carbohydrate content) provides a plausible explanation of the increased uptake of nitrogen compounds by the fertilized boll, and, judging from the consistent response of residual N in carpel and ovule, that fraction may be the mobile compound. There may also be an increased ease of entry.

We may summarize the effects of fertilization on the uptake of material and on sap concentration as follows:—The behaviour of a compound responsible for the increased uptake of a substance, carbohydrate or nitrogen, by ovule and carpel should fulfill one of the following conditions.

Rate of Utilization.	Ease of Entry.	Sap Concentration.
1. Increase	{ a. Increase	{ I. Increase
		{ II. No change
	{ b. No change	{ III. Decrease
		Decrease
2. No change	Increase	Increase

As estimates of rate of utilization as well as information as to the sap concentrations of the various compounds are available, we can determine to what extent any one compound or compounds satisfies the above con-

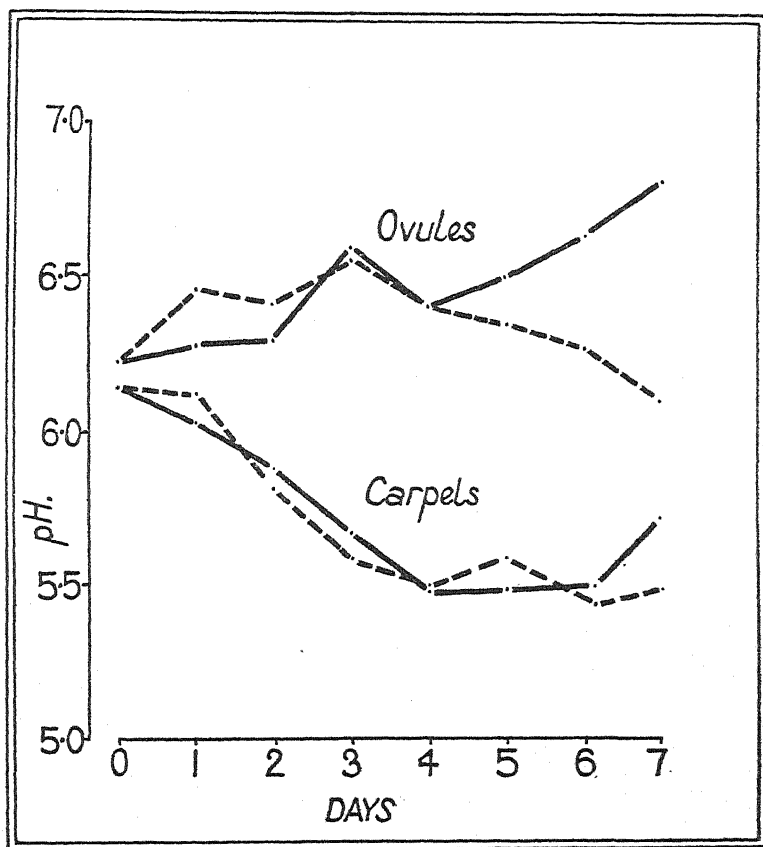


FIG. 7. Hydrogen-ion concentration in sap of Normal group (continuous line), and Unfertilized group (broken line).

ditions. For carbohydrate uptake sucrose fulfils the conditions α II in both ovule and carpel, and as there is a marked sucrose gradient from sieve-tube to ovule and carpel, it appears probable that the effect of fertilization on carbohydrate uptake is both an increased rate of utilization of sucrose and an increase in its ease of entry. For nitrogen uptake residual N fulfils conditions β or α III in ovule and carpel, and as there is probably a considerable gradient in this fraction between the sieve-tube on the one hand and the ovule and carpel on the other, it seems probable that the effect of fertilization is to bring about mainly an increase in the rate of utilization of residual N. The results, therefore, support the suggestion that carbohydrates

enter the boll as sucrose, and that nitrogen escapes from the sieve-tubes as residual N.

Hydrogen-ion concentration. The hydrogen-ion concentrations in the sap of fertilized and non-fertilized bolls are shown in Fig. 7. In the ovule the pH rises somewhat irregularly in both groups up to day 4. After that it continues to rise in the Normal group but falls in the Unfertilized. In the carpels no difference between the two groups can be detected until day 6, when the Unfertilized group falls below the Normal. The pH in the bark varied slightly about a mean of 5.58.

SECTION 4. SUMMARY.

A 1. On the basis of earlier work, a tentative picture of the transport of carbohydrates and organic nitrogen is put forward as follows. For nitrogen as well as for carbohydrate transport there seems to be a gradient basis. For carbohydrates the head in the leaf is apparently reducing sugars, while for nitrogen the head is residual N. Within the sieve-tubes all the soluble carbohydrates and all the labile forms of nitrogen, including protein, should contribute to longitudinal transport, the part played by each depending on the effective concentration gradient maintained, and probably also on the diffusion constant. The mechanism (possibly protoplasmic streaming) which is responsible for accelerating diffusion along the sieve-tubes should act impartially on all materials that are free to move. Movement from the sieve-tubes into other tissues and vice versa, is presumably confined to crystalloid substances, and in the case of nitrogen there is some evidence suggesting that residual N is the most important fraction. The rate of movement out of the sieve-tubes should depend on the effective gradient of exit maintained.

2. In the present paper we consider this picture of transport in relation to some aspects of the uptake of material by the boll.

B 1. In the first experiment a study is made of the effect of removal of flower-buds and bolls on the carbohydrate and nitrogen content of the leaves and the stem tissues.

2. Removal of flower-buds and bolls was followed by an increase in concentration of carbohydrates and of nitrogen, not only in the bark, but also in the wood and the leaf. Removal of a 'sink', the flower-buds and bolls, is thus similar in its effects to the isolation of another 'sink', the roots, by ringing the main-axis at ground level; and the results confirm the general conception of a gradient basis for the transport of nitrogen and carbohydrates.

3. The percentage increase in nitrogen content was greater than that in carbohydrate content.

4. Polysaccharides account for a large part of the carbohydrate response, but total sugars also show a well-marked response, mainly due to

sucrose. Protein N responds particularly in the leaves and in the upper region of the bark. Crystalloid N shows a well-marked response in all regions, and the percentage increase in crystalloid N is, in general, greater than that in protein N. In the leaves the crystalloid N response is mainly residual N; residual N responds also in bark and wood, but except in the upper region of the wood the increases are small. The greater part of the crystalloid N response in the stem-tissues is due to asparagine. Nitrates are almost unchanged in the leaves, but show a fall in bark and wood of the upper region.

The results are in most respects similar to those obtained on ringing the stem near its base.

C 1. In the second experiment an attempt was made to elucidate the method of carbohydrate and nitrogen uptake by the boll. The uptake of carbohydrates and of nitrogen, and the drift of sap concentrations, were followed for seven days in fertilized and unfertilized bolls. Ovules and carpels were handled separately.

2. A marked difference as between fertilized and unfertilized bolls, in uptake of carbohydrates and nitrogen, and a definite divergence in the march of sap concentrations of certain compounds, became apparent in both ovules and carpels on the fourth day after anthesis.

3. Two types of change are distinguished as likely to lead to the increased uptake by the fertilized bolls: (1) an increased *rate of utilization* of the mobile compounds (i. e. compounds that enter the ovules and carpels from the sieve-tubes); (2) an increased *ease of entry* for these compounds. In the first case the concentration of the mobile compound in the growing organ diminishes, the gradient of entry steepens, and the rate of uptake increases. In the second case the rate of movement for unit gradient of entry increases, the concentration in the organ increases, and transformation (growth) becomes in consequence more rapid. If both factors operate there may be no change in concentration.

4. Ease of entry cannot be exactly measured, since we do not know the effective gradients of entry. But from (1) the amount of carbohydrate (or nitrogen) transformed during any time interval, and (2) the mean amount present during that time interval, of any compound that is assumed to be the mobile compound, estimates may be obtained of rates of utilization. *Utilization indices* can be calculated in this way for each of the compounds studied; and information is also available in each case as to the change of sap concentration during growth.

5. Applying these criteria to the results obtained for the fertilized and unfertilized bolls, it seems probable that the increased uptake of carbohydrate by the fertilized ovules and carpels is due to an increased ease of entry of sucrose, coupled with an increased rate of utilization. The concentration of sucrose remains low during growth, and maintains approximately

the same level in fertilized and unfertilized bolls. In the case of nitrogen, the results strongly suggest that the greater uptake by the fertilized ovules and carpels is due to a higher rate of utilization of crystalloid N, producing a lower concentration and therefore a steeper gradient into the ovules and carpels. In the ovules this increase in rate of utilization and decrease in concentration is shown by asparagine and residual N, but in the carpels by residual N only. From a consideration of the gradients into the boll during growth it seems probable that for both ovules and carpels the residual N fraction, rather than asparagine, is the form in which organic nitrogen enters from the sieve-tubes. In addition to an increased rate of utilization of residual N there may also be some increase in ease of entry.

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Storage and Other Carbohydrates in a Natal Succulent and a Natal Geophyte and their Behaviour Before, During, and After the Winter Resting Season.

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INTRODUCTION.

THE following pages contain an account of certain aspects of the physiological behaviour of two representative types of the sub-tropical flora of Natal. The research forms part of a general scheme initiated some years ago by the senior author. After the vegetation of Natal had been analysed and classified by him into its various plant communities, attention was directed to its relationship with the vegetation of outside areas. The question of the origin and evolutionary history of the South African flora as a whole was discussed, first of all in 1922 (1) and, later, in more detail, in 1925 (2). In 1927 (3) it was shown that the principles first analysed with regard to the South African flora throw a good deal of light on the ecological evolution of angiosperms as a whole, and that the primitive angiosperm types are probably to be found in the moist tropical forests under conditions which have remained more or less uniform and unchanged since the rise of flowering plants. As might be expected, the moist tropical floras of Central Africa, of South America, and the Indo-Malayan regions show interesting and, in many respects, fairly close connexions with one another. In these ancient moist tropical reservoirs of plant life, differentiation during an enormous period of time, under relatively constant conditions, has resulted in the production of a great number of forest types of growth form.

Response to colder conditions, which began apparently at the close of Cretaceous times, has resulted in the production of the temperate types, but a fuller discussion hardly falls within the scope of the present paper.

Response to drier conditions may also have begun at an early date, but it probably became pronounced only about the middle of the Tertiary or later. In Africa the evidence is overwhelmingly strong in favour of the view that the drier sub-tropical flora is a very highly evolved type phylogenetically. The Karroo flora, it may be claimed, represents the very latest experiments on the part of nature in plant evolution. There is hardly a single element in it which is not an advanced type.

From the standpoint of pure systematics it is all very fascinating; for the student of plant morphology the problems are practically endless, and much useful work has already been accomplished by making use of the comparative method in those directions. Interesting though the results are that can be obtained by the methods of comparative morphology, it has been felt that a still deeper insight can be obtained into the evolutionary history of plants by investigating the underlying basic physiological processes. Morphological changes are not in themselves sufficient to account for the production of types capable of living in dry sub-tropical regions. Further, it might be urged that morphological changes are the result of physiological changes, that changes of function precede changes of form, and that morphological changes cannot be fully understood until we know more about plant behaviour.

A sub-tropical flora has to survive a periodical, adverse, dry season, and response to this adverse dry season has been the chief factor influencing the evolution of sub-tropical floras. Provision has to be made for the existence of vegetation during these periods and for the renewal of activity on the return of better conditions.

The external morphology of evergreen perennials does not indicate how they respond to dry seasons. One must look to internal changes, such as fluctuations in the content of carbohydrates, particularly colloidal carbohydrates, to help solve the problem. Spoehr (17) has found that in cacti pentosans increase with low-water content and high temperatures, while we, if we may anticipate a little, have found that, in succulents, low-water content is more important than temperature in causing the increased production of pentosans and that, in all probability, colloidal hexosans behave in the same way as colloidal pentosans. There seems little reason to doubt that the various properties of these colloids play an important part in adapting plants to dry conditions, and that the production of carbohydrate colloids influences the water relationship of succulents and probably, to a lesser extent, of other types. It is, however, by no means clear exactly how the colloids serve such a useful purpose. In deciduous plants there is an obvious response to adverse seasons, but even the phenomenon of leaf-fall does not necessarily completely explain their survival during dry seasons. Even in these plants there is always the possibility of various biochemical responses to adverse conditions. Apart from such biochemical changes in deciduous-

leaved plants there are other biochemical changes connected with leaf-fall itself.

At the end of the resting season, when plant life again becomes active, new leaves are formed, and the plant very often begins to flower. Flowering, however, does not always occur in spring. This brings one to the question of food storage. How much food do plants store, and how much of this storage is used during spring growth? Storage would appear to be particularly necessary in deciduous types, and especially so in such types as geophytes, which may produce new leaves and flowers before the first rains fall. Advanced sub-tropical types very often store much carbohydrate. In geophytes the underground storage organs may become very large in proportion to the rest of the plant. Succulents also have large food reserves as a rule. Strangely enough, chemical analyses showed that little of the carbohydrate food reserves were drawn upon for spring growth. Microchemical examinations indicated that other succulents behaved similarly.

CLIMATE.

The research was carried out on plants growing in the grounds of the Natal University College at Pietermaritzburg. The climate is of the sub-tropical type with wet summers and dry winters. The bulk of the annual rainfall occurs during the hot period between October and March, while the combined effect of drought and cold produces a decided resting period for the rest of the year. At the beginning of spring the weather generally becomes warmer, but little rain falls. In spite of the drought this return of warmth causes the production of new spring growth in many types, particularly geophytes.

THE PLANTS.

Portulacaria afra, Jacq, was the succulent type studied and *Hypoxis rooperi*, Moore, the geophytic type.

Portulacaria afra is a shrubby, much-branched tree with small succulent leaves. It is a common member of the xerophytic, thorn-veld areas, where it may dominate. The tree is spineless. The leaves are opposite, obovate-roundish, succulent but nearly flat, and small (up to $\frac{3}{4}$ -in. long and $\frac{1}{2}$ -in. broad). A transverse section of the leaf does not reveal any very specialized tissues such as are found in many other succulents. But although the leaves show little differentiation in appearance and anatomy, they are very succulent, and their water content varies from 78 per cent. to 92 per cent. of their fresh weight. The leaves have no starch grains when picked early in the morning, and seem to have no storage function as far as carbohydrates are concerned. This is borne out by a study of the diurnal change in

carbohydrate content. The amount of carbohydrate hydrolysable by dilute acid increases by about 50 per cent. between 6.30 a. m. and 5 p. m., and shows a corresponding decrease during the night. The relatively large daily increase, representing the amount synthesized, less the amount translocated or respired, would indicate that little, if any, of the products of photosynthesis are retained by the leaf. The storage of carbohydrates seems to be confined to the twigs and branches, where the storage regions are packed with starch.

Although many species of succulents, including cacti, were available for experimental purposes, *Portulacaria afra* was selected, since it is almost ideal for experimental purposes. By analysing leaves picked from time to time in the early morning, changes in the content of the important colloidal polysaccharides were found, and the interpretation of results was not confused by including storage carbohydrates and the products of photosynthesis. By analysing small twigs from time to time changes in the content of storage carbohydrates were found, since this type of carbohydrate predominates in those regions. Thus by selecting the right type of material for experiment one is able to differentiate between the seasonal changes in two very distinct types of polysaccharides, that is, the storage elements and the colloidal types.

Hypoxis rooperi is a common grass-veld geophyte. The underground portion consists of a hard, perennial corm with conspicuous gum canals bearing long fleshy roots. The corm may become very large relative to the rest of the plant, and is very rich in carbohydrates. The corm bears many thin, membranous leaves, but the number of leaves does not bear any direct relationship to the size of the corm. Small corms generally bear few leaves, but in more mature corms the number of leaves remains rather uniform, in spite of great differences in the size of the corms.

Hypoxis rooperi was selected because it is rather a primitive type. As will be seen later, this fact is advantageous for experimental purposes in many respects, although it has certain disadvantages. The corms were analysed from time to time to observe the changes in the storage organ. The leaves when present were also analysed at intervals to throw some light on various biochemical problems.

THE SELECTION AND PREPARATION OF MATERIAL FOR ANALYSIS.

To reduce the large errors which may be incurred by the use of samples which are not thoroughly representative, large samples were collected under similar conditions throughout the year. We aimed at trying to reduce errors to such an extent that the necessity for their exact estimation was obviated.

Portulacaria afra leaves were picked in samples of about 1,000 from

a single tree, to which attention was confined so as to avoid errors due to differences between tree and tree. Leaves were collected from one side of the tree (the sunny, north side). An analysis of leaves at the beginning of May showed that the pentosan and hexosan contents on the north side were respectively 49.9 per cent. and 45.5 per cent. higher than the corresponding amounts on the south side (see Table X). Samples were collected just before sunrise, at roughly the same height from the ground throughout the investigations.

Portulacaria afra twigs were collected just before sunrise from the same side of the tree. They were freed from leaves before drying.

Hypoxis rooperi corms weighing between 30 and 75 gm. were collected in samples of fifteen. Small corms were collected because, although the chemical composition of the corms varies only slightly with size, the smaller samples are more uniform and have no localized production of cork. Moreover, it was thought that the draining effect of spring growth on the carbohydrate reserves would be more apparent in small corms. After freeing these corms from roots, leaves, and as much soil as possible, they were cut up into small chips and dried. It was impossible to remove all adhering soil particles, so samples of dry, powdered corm material were ashed, and carbohydrate content was expressed in terms of dry, combustible material. This is not very different from expressing results in terms of dry weight.

Samples of *Hypoxis rooperi* leaves were carefully freed from the corms and, in autumn, any dead areas were removed before drying.

In drying the material the samples were put in a dish fitted with a cover and placed in an oven at 100° C. for about forty minutes. The cover was then removed and the temperature of the oven lowered to about 86° C. When the material was almost dry, the temperature was again raised to 100° C. to drive off the last traces of water. The material was then brought to a fine uniform powder in a mill, and was stored in a stoppered jar.

Small portions of the fresh material were dried for water-content determinations. These were done in duplicate, and results agreed closely.

ANALYTICAL METHODS.

The following classes of carbohydrate were estimated: pentosans, hexosans, sucrose, and monosaccharides. The figures for the monosaccharide class are not very accurate, since this class included both hexoses and pentoses, which reduce Fehling's solution to different degrees. The figures would also be affected by the small amounts of maltose which were not separately estimated. For our purposes this procedure was considered sufficiently accurate.

A suitable, weighed quantity of powdered plant material was mixed with 2 grm. of powdered calcium carbonate and twice extracted on a water-bath for three hours under a reflux condenser, with 200 c.c. of 90 per cent. alcohol, and filtered hot. The filtrate, which contains the monosaccharides and disaccharides, was freed from alcohol by distillation on a water-bath under reduced pressure. The residue was taken up with water, clarified with normal lead acetate, and the clarified residue delead with a slight excess of sodium carbonate. The resulting liquid was neutralized and made up to known volume. An aliquot was used for the estimation of reducing sugars, while another aliquot was inverted and the increase in reducing powers due to sucrose was estimated. Inversion was done under Herzfeld conditions, except that an extra equivalent of acid was added for every equivalent of acetate, derived from the lead acetate, which was calculated to be present in the aliquot.

Inversion under Herzfeld conditions scarcely affects maltose. The amount of maltose present was small. Aliquots were hydrolysed with 10 per cent. citric acid, which does not hydrolyse maltose, and to completion with dilute hydrochloric acid. Complete hydrolysis with hydrochloric acid gave reducing values which were sometimes higher but often lower than those given by aliquots hydrolysed by citric acid. Lower values were probably due to the destructive effect of hydrochloric acid on the hexoses and pentoses. Davis and Daish (7) think that it is impossible to hydrolyse maltose completely in the presence of sucrose without destroying a considerable amount of hexoses.

The estimation of polysaccharides was complicated by the fact that the mucilaginous nature of some of the material prohibited the use of enzymes, such as diastase, for hydrolysis. Acids had to be used, in which case both hexoses and pentoses occurred as products of hydrolysis.

A weighed sample of powdered plant material was hydrolysed for $4\frac{1}{4}$ hours with 200 c.c. of 1.1 per cent. hydrochloric acid on a boiling water bath. This was filtered and the residue washed with hot water. The filtrate and washings were clarified with normal lead acetate, except in the case of *Hypoxis rooperi* corms, and delead in the presence of a little mineral acid with hydrogen sulphide. A little excess sodium acetate was added to remove mineral acid, and the solution, which was feebly acid owing to the presence of acetic acid and acetate ions, was evaporated to remove all hydrogen sulphide. It was then neutralized and made up to known volume. In the case of *Hypoxis rooperi* corms, which are very rich in carbohydrates, the products of hydrolysis were not clarified, since this was found to be unnecessary. The reducing value of the hydrolysed solution was then estimated with Fehling's solution.

Dilute hydrochloric acid was used because, according to Spoehr, it has less effect on cellulose than other acids of the same ionic concentration.

The time of hydrolysis ($4\frac{1}{4}$ hours) was fixed after a good deal of experimenting. By hydrolysing fresh samples of powdered plant material for different lengths of time, it was found that after roughly $3\frac{1}{2}$ to $3\frac{3}{4}$ hours all the more easily hydrolysable polysaccharides were hydrolysed. The time was lengthened to $4\frac{1}{4}$ hours to introduce a margin of safety. There is no *a priori* reason why, say, $3\frac{1}{2}$ hours hydrolysis should be sufficient for spring samples, although this time was sufficient for autumn samples. The lengthening of this period seemed justified by the great changes in carbohydrate content during the course of the year and by the small, possibly fluctuating, buffering capacity of the plant material. This policy has two big disadvantages; the amount of cellulose hydrolysed is increased, and more sugars, particularly pentoses, are destroyed by the acid. In both cases, however, no new errors are introduced, but errors already present are increased. These errors would presumably occur to approximately the same extent in successive analyses, so results would be comparable. To counteract fluctuations in hydrogen-ion content, due to the buffering of the mineral acid, the volume of acid used was very large. Under these circumstances the hydrogen ions removed do not affect the hydrogen-ion concentration to any serious extent.

The reducing value of the hydrolysed solution represents the sum of the reducing powers of monosaccharides, inverted sucrose, and hydrolysed polysaccharides. The reducing value of hydrolysed polysaccharides can be calculated by difference. The amount of hydrolysed pentosans, and hence the reducing power of the hydrolysed pentosans, was estimated by the Kröber method in the manner to be described. These data are sufficient for the calculation of the amount of hexosan. The scheme may be summarized as follows:—

- A. The reduction by uninverted alcoholic extract (monosaccharides).
- B. The reduction by inverted alcoholic extract.
- $C = B - A$. The reduction by inverted sucrose.
- D. The reduction by material hydrolysed by 1.1 per cent. acid.
- $E = D - B$. The reduction by hydrolysed polysaccharides (pentosans and hexosans).
- F. The reduction by hydrolysed pentosans (calculated from the pentosan figure given by Kröber method).
- $G = E - F$. The reduction by hydrolysed hexosans.

The reducing powers of the various sugar solutions were determined by boiling with excess Fehling's solution (Soxhlet's modification) and determining the amount of unreduced copper. The procedure of copper reduction is purely empirical, and the amount of oxygen consumed has no stoichiometric relation to the amount of reducing sugar employed. The whole process, including the approximate concentration of sugar solution, the concentration of the Fehling's solution, the time required to bring the

solution to the boil (four minutes), and the time of boiling (two minutes), was carefully standardized.

Excess Fehling's solution was determined in two ways.

In the estimation of monosaccharides and disaccharides, the hot Fehling's solution mixture, containing cuprous oxide and excess unreduced copper, was immediately cooled and filtered. An aliquot of the filtrate was made slightly acid with sulphuric acid, 1 to 1.5 c.c. of concentrated acid being used for every 20 c.c. of Fehling's solution originally present in the aliquot. The copper was then determined by the iodimetric method.

In estimating total carbohydrates, where the amount of nitrate is very small in proportion to the amount of carbohydrate, a much more rapid, and probably more accurate, method was used. The method was based on that given by Knecht and Hibbert (11). If, to an acidified copper solution, one adds pure ferrous ammonium sulphate and excess potassium sulphocyanide, the copper is reduced to the cuprous form, which precipitates out as white cuprous sulphocyanide, and an equivalent amount of ferrous salt is oxidized to the ferric condition, giving a blood-red colour with the excess sulphocyanide. A standard titanous chloride solution is run in until all the iron is reduced to the ferrous condition, as shown by the disappearance of colour from the solution. The end-point is remarkably sharp, and provided the usual precautions attached to titanous chloride titrations are observed the method is very accurate. Knecht and Hibbert applied this method to the estimation of excess unreduced copper in the Fehling's solution after filtering off the cuprous oxide in the usual way and acidifying an aliquot of the filtrate with hydrochloric acid. We found, however, that it was quite unnecessary to filter off the cuprous oxide. After boiling the Fehling's solution with the sugar solution in the usual way, the mixture was cooled immediately and acidified with hydrochloric acid without first filtering off the cuprous oxide. A freshly made solution of excess ferrous ammonium sulphate, excess sulphocyanide, and a little acid was then added, and titanous chloride run in until the red colour disappeared. The amount of titanous chloride added is exactly equivalent to the amount of unreduced, excess copper in the Fehling's solution. It should be noted that the mixture of the ferrous salt and sulphocyanide should not be pink. If it is pink, just sufficient dilute titanous chloride is added drop by drop until the mixture is colourless, and can then be added to the acid copper solution. The theory of the whole process of estimating excess copper in the presence of cuprous oxide is that, on acidifying such a mixture, the cuprous oxide is turned into cuprous salt, and the excess copper is changed into cupric salt. On adding the ferrous salt and sulphocyanide the cuprous salt is turned into cuprous sulphocyanide, which is very insoluble, and is therefore removed from the solution, while the cupric salt behaves in the manner already outlined.

This new method was tested on pure sugar solutions and found to be accurate. Since it obviates the necessity of filtering off the cuprous oxide it is not exposed to the danger of the re-oxidation of some cuprous oxide by the air, and is, in addition, exceptionally rapid. It cannot be used in the presence of nitrates, so it was only used for the estimation of total carbohydrates where the amount of nitrate is proportionally small. It was to remove such oxidizing agents that in total carbohydrate examinations the sugar solutions were deoxygenated with hydrogen sulphide in a mineral acid solution. Whether this treatment removed oxidizing agents we cannot say, but it is certain that little, or no, nitrates were left because results obtained by the titanous chloride method agreed exactly with those by the longer iodimetric method.

Expressing factors in terms of Fehling's copper sulphate solution, 1 c.c. of this solution is equivalent to 0.01 grm. glucose, 0.0095 grm. cane sugar, 0.0090 grm. hexosan, and 0.0080 grm. pentosan. Monosaccharides were estimated by using the glucose factor in spite of the fact that pentoses were present.

Pentosans were estimated by the Kröber-Tollens method. We need not enter into the controversy between the users of this method and the fermentation method; both seem to be sufficiently accurate if precautions are taken. It was in view of this controversy that we used the Kröber method, because Spoehr used the rival fermentation method, and it seemed desirable to try to confirm his results by another method, and hence remove any doubts that existed on the subject.

The estimation of pentosans by the Kröber method, and the calculation of hexosans from the amount of pentosans and the total reducing powers of the polysaccharides is not straightforward, and special precautions have to be taken to ensure that only those pentosans hydrolysed by dilute acid in the given time ($4\frac{1}{2}$ hours) are estimated. Besides pentose-containing substances which are not strictly pentosans, the pentosans can be divided into two classes—those easily hydrolysed with dilute acid and those which are more or less resistant to dilute acid. In lignocelluloses the latter class has been the subject of controversy. Wise (20), among others, holds that these pentosans are adsorbed on the surface of the cellulose, while Schorger (16) and others think that they are held in a state of chemical combination.

Pentosans hydrolysed by 1.1 per cent. hydrochloric acid in $4\frac{1}{2}$ hours were estimated as the difference between total pentosans and those not hydrolysed by the dilute acid in $4\frac{1}{2}$ hours.

Total pentosans were estimated by extracting the plant powder with 95 per cent. alcohol under a reflux condenser on a water-bath to remove pentoses. The residue was filtered off on a Gooch crucible, washed with hot alcohol, dried and treated, together with the asbestos, with hydrochloric

acid under Kröber conditions. The furfural in the distillate was precipitated as furfural phloroglucide, which was treated with alcohol in the usual way to remove alcohol-soluble phloroglucides.

Residual pentosans (i. e. those not hydrolysed to pentoses in $4\frac{1}{4}$ hours by the dilute acid) were estimated as follows: A fresh sample of plant material was hydrolysed for $4\frac{1}{4}$ hours with 1.1 per cent. hydrochloric acid without previously extracting the pentoses. Without filtering off the residue the mass was neutralized and evaporated to about 10 c.c., and 100 c.c. of 95 per cent. alcohol were added slowly with constant stirring. The alcoholic mass was then allowed to stand overnight, and was finally boiled for three hours on a water-bath under a reflux condenser to dissolve pentoses. The residue was filtered off, extracted again with 85 per cent. alcohol, filtered, washed with hot alcohol, dried and treated under Kröber conditions to estimate pentosans.

The difference between these two pentosan estimations gives the amount of pentosans hydrolysable by dilute acid in $4\frac{1}{4}$ hours. The use of alcohol to separate hydrolysed from unhydrolysed pentosans is essential, although this has been done by few workers, if any at all. It would appear that after hydrolysis with dilute acid a certain amount of unhydrolysed pentosans occur in a colloidal state in the aqueous extract. The removal of these pentosans from solution by alcohol is necessary, and failure to do this is probably largely the reason for the often great differences between pentosan figures obtained by the Kröber and by the fermentation methods. If alcohol is not used the figure for hydrolysable pentosan is too high.

The indirect method of estimating hydrolysable pentosans as the difference between total pentosans and residual pentosans not hydrolysed by dilute acid gives more accurate results, since it is not affected by the amount of pentoses destroyed during hydrolysis with dilute acid. Under this scheme of analysis any errors due to this cause are thrown on to the hexosan figure, which is consequently too low.

Judging from the rotary powers of fermented portions hydrolysed, the pentosans in the species used in this research were mixtures of xylan and araban. This is unfortunate since xylose and arabinose have different furfural yielding capacities.

ERRORS.

Errors may be incurred through faulty analytical methods and through the collection of samples which are not thoroughly representative.

Most of the faults in the analytical methods have already been discussed while dealing with the various methods. It is generally difficult to give a figure for the probable magnitude of these errors; all one can ascertain is that similar errors will occur throughout, and that results will consequently

be comparable. Since results were used for purposes of comparison, this is essential. Various samples of plant material were analysed several times, and results were shown to agree very well. A 4 per cent. difference between analyses of the same material were occasionally met, but differences were usually much smaller, being often below 1 per cent. The smallest differences were found between analyses for polysaccharides.

Figures can be given in the case of the maximum possible error introduced by the pentosans being a mixture of xylan and araban. The pentosan figure given is the average between the amounts of araban and xylan, which give a definite amount of furfural. 1.0 grm. pentosan may mean 1.09 grm. araban or 0.91 grm. xylan. The figures for hexosans would be similarly affected. This source of error does not affect the figure for total polysaccharides, since too high a pentosan figure means a correspondingly low hexosan figure, and vice versa.

With regard to errors due to the use of samples which are not properly representative the policy pursued was to increase the size of the sample to reduce the error. These errors are the most troublesome since, unlike analytical errors, they do not occur to a more or less equal degree throughout, and results are not comparable.

Hypoxis rooperi corms. Owing to the fact that *Hypoxis* plants do not all begin to enter the winter resting period at the same time, but show slight individual differences, it was thought that there might be a seasonal change in the amount of error, the smallest errors occurring, presumably, when all leaves are entirely green, or when all plants are resting. For this reason samples of corms were collected in duplicate on three separate occasions (on March 23, when leaves were all green; on May 1, when leaves were dying; and on June 14, when all leaves were dead), and the greatest variation was found on May 1 (see Table I).

TABLE I.

(Analyses Expressed per cent. Dry Combustible Material.)

	Sample A.	Sample B.	$\frac{(A-B) \times 100}{A}$
Water content . .	59.74	59.81	
Total carbohydrates .	59.8	58.0	+3.0
Total polysaccharides*	55.2	53.4	+3.3
Total soluble sugars .	4.64	4.59	+1.1
Pentosans	3.20	3.23	-0.9
Hexosans	52.0	50.1	+3.7
Disaccharides . .	2.66	2.77	-4.1
Monosaccharides .	1.98	1.82	+8.0

Hypoxis rooperi leaves. The number of leaves collected in a sample was so large that it was thought unnecessary to estimate differences between samples.

Portulacaria afra leaves. Differences between samples picked at the same time were quite negligible, as is to be expected in samples of 1,000 leaves picked from one side of the tree only.

There is another source of error to be reckoned with in dealing with seasonal variations. When determining seasonal variations in, say, carbohydrate content, it is out of the question to collect samples daily. A sample is collected, say, once a month on the assumption that the sample is representative not only of the day on which it is collected, but also more or less representative of days just before and after. Is this assumption correct? Samples were collected at the usual time on two consecutive days. This was done once in May and once in June. In the June experiment the only difference between the two days was a slight (unmeasured) change of temperature. On the colder day the water content was 0.3 per cent. less, while the pentosan and hexosan contents were respectively 8 per cent. and 9 per cent. higher than the corresponding figures for the warmer day. The May experiment showed a smaller change, and here again an increase of pentosan content was accompanied by an increase in hexosan content. The importance of this fact will be pointed out later. It is doubtful how much stress should be laid on this type of error. It shows clearly that one cannot foretell the carbohydrate content in any particular calendar month, yet this type of error does not appear to be serious if one considers seasonal changes in the light of changes of environment, &c., without paying much attention to the calendar. (For this reason records were kept, of soil moisture content at a depth of 8 in. and of soil temperature at a depth of 4 in.). The carbohydrate content on any particular day would be made up of previous daily increases and decreases, and would probably be representative of the particular day, if we judge this day from the prevailing environmental and internal conditions. This speculation does not seem unreasonable in view of the fact that the carbohydrates can change with remarkable rapidity.

Portulacaria afra twigs. Differences between analyses of three samples collected at the same time were more appreciable here. The difference between the two most different samples was 6 per cent. for hexosan, 4 per cent. for pentosan, and 4 per cent. for monosaccharides and disaccharides.

SEASONAL CHANGES IN THE CARBOHYDRATE CONTENT OF *HYPOXIS ROOPERI* LEAVES.

Four analyses are given in Table II. The first column gives the content of green autumn leaves on March 23. On May 1 the leaves were beginning to die, but as far as possible all dead portions were removed before analysis. The third column gives the content of dead leaves. The last column gives the carbohydrate content of young, not fully developed, spring leaves.

TABLE II.

Seasonal Variation in Carbohydrate Content of Hypoxis rooperi leaves.

(Analysis Expressed as Percentages of Dry Weight.)

	March 23.	May 1.	May 19.	October 3.
Water content . . .	79.8	73.0		82.3
Total carbohydrates . . .	12.3	12.9		15.4
Total polysaccharides . . .	10.9	11.6	10.7	13.0
Total soluble sugars . . .	1.40	1.31		2.42
Pentosans . . .	5.84	6.64	6.66	9.51
Hexosans . . .	5.01	4.93	4.00	3.46
Disaccharides . . .	0.59	0.55		1.12
Monosaccharides . . .	0.81	0.76		1.30
Moisture content of soil . . .	19.8%	18.1%		23.1%
Soil temperature . . .	25.5° C.	22.2° C.		19.1° C.
Remarks . . .	Leaves green.	Leaves dying.	Leaves dead.	Spring leaves.

Residual pentosans (not hydrolysed by 1.1 per cent. hydrochloric acid in $4\frac{1}{4}$ hours) increased from 4.56 per cent. in green autumn leaves to 4.60 per cent. in dead leaves, while the figure in spring was 4.43 per cent.

The figures for the living and dead autumn leaves show that changes in carbohydrate content are gradual, and death does not bring about any great changes in carbohydrate composition.

A comparison of living and dead leaves is interesting. Hexosans tend to decrease, but this may be due to the fact that the green leaves contained products of photosynthesis, because, owing to bad planning, the samples of autumn leaves were not picked before sunrise in the case of *Hypoxis rooperi*; pentosans tend to increase. This might fit in with the ideas of those who regard pentosans as inert, and, possibly, waste products, but the fact can also be explained by assuming that this class is of vital importance. Any important class could not be withdrawn from the living cells without causing the death of these cells. It is therefore unlikely that they could be removed to any large extent. Lastly, one could take the view that the plant does not necessarily prepare for the death of the leaves by reclaiming as much food material as possible.

The carbohydrates of the spring leaves are remarkable. Externally the leaves in spring are not very different from those in autumn. Water content and residual pentosans are similar. Soluble sugars are more abundant, probably because of the more active nature of spring leaves. But polysaccharides are the most interesting class. Hexosans are low, partly because the leaves were picked early in the morning. Pentosans are remarkably high, probably because of the importance of colloids in young cells with small vacuoles. If so, it would tend to show that internally the

cells do not mature as quickly as the external appearances of the leaves would lead one to believe.

THE SEASONAL CHANGES IN CARBOHYDRATE CONTENT OF
HYPONIS ROOPERI CORMS.

Analyses were made from autumn to spring. The first two samples (collected on March 23 and May 1) had leaves, although in the second sample the leaves were approaching death. The samples collected on June 14 and August 13 were without leaves. On August 28 the samples consisted of corms whose buds were developing, although they were not yet green. The last sample bore new spring leaves. The analyses are shown in Table III. Each of the first three columns represents the average of two analyses.

TABLE III.

Seasonal Changes in Carbohydrate Content of Hypoxis rooperi Corms.

(Analysis Expressed as Percentages of Dry Combustible Material.)

	Mar. 23.	May 1.	June 14.	Aug. 13.	Aug. 28.	Oct. 3.
Water content . . .	63.9	59.7	63.3	60.6	63.4	66.3
Total carbohydrates . . .	58.4	59.0	64.7	63.1	65.5	66.6
Total polysaccharides . . .	51.4	54.4	60.5	59.0	59.5	59.1
Total soluble sugars . . .	7.0	4.6	4.2	4.1	6.0	7.5
Pentosans . . .	3.06	3.25	3.26	3.87	3.10	3.19
Hexosans . . .	48.3	51.1	57.2	55.1	56.4	55.9
Disaccharides . . .	4.0	2.7	2.6	2.7	3.8	4.7
Monosaccharides . . .	3.0	1.9	1.6	1.4	2.2	2.8
Water content of soil . . .	19.8 %	18.1 %	16.3 %	14.6 %	15.1 %	23.1 %
Soil temperature . . .	26.5° C.	22.2° C.	17.0° C.	16.8° C.	20.0° C.	19.1° C.
Remarks . . .	Leaves green.	Leaves dying.	Plants resting.	Plants resting.	Buds developing.	Spring activity.

Residual pentosans varied within small limits, about approximately 1.2 per cent. dry combustible material.

The tables show that, while certain changes are brought about by the death of leaves and by the production of leaves in spring, these changes are not great. The constancy in carbohydrate composition, in spite of morphological and other changes, is worth noting.

Both classes of soluble sugars were less in amount during the resting season. The regularity of the changes in these two classes, in spite of fluctuations in water content, suggests that changes in activity are more important than changes in water content in bringing about changes of sugar content. If one could be sure of this fact, one would suggest that physiological changes precede morphological changes because, as regards the content of soluble sugars, the sample with green but moribund leaves collected

on May 1 is typical of the resting condition, while the sample with slightly developed buds collected on August 28 is approaching the spring condition.

Changes in pentosan content seem to be influenced both by water content and activity. The figure for October 3 is higher than one would expect. It is not at all clear whether pentosans increase with aridity in geophytes as they do in succulents. The subject will be briefly discussed at the conclusion of this paper.

The changes in hexosan content or in the total amount of carbohydrate are very important, since they are directly connected with the problem of storage. They are not depleted by spring growth—in fact they are high in amount at this time. This would indicate that very little, if any, of the reserve carbohydrates are used to help in the renewal of growth. Nor can one doubt the significance of the figures given in Table III. It might be argued that, since the corms have practically no fibre, other classes of food reserves, e.g. proteins and fats, may be drawn upon to an equal or greater extent than are the carbohydrates, so that the relative amount of carbohydrate in the corm is not lessened, although the actual amount in the corm might be diminished. If this were so, one would expect the corms to be shrivelled or show other signs of having lost some of their contents. This is not so.

The seasonal changes in carbohydrate content of both the leaves and corms of *Hypoxis rooperi* throw some light on the evolutionary development of deciduous geophytes. *Hypoxis rooperi* is rather an old and unspecialized type. The reasons for this statement seem conclusive when taken together, although each of them stated singly would carry little weight. Among the Amaryllidaceae it is fairly primitive, since it has only a half-sunken ovary. In growth form it is not specialized when compared with highly evolved geophytes found in the Karroo and elsewhere. Vegetative and reproductive growth take place rather late in spring, and seem to be fairly dependent on rainfall; many geophytes become active very early in spring and are remarkably independent of rainfall for spring activity. It is rather mesophytic and, unlike most geophytes, can withstand a large amount of shading by the mesophytic grasses with which it is associated. These facts are sufficient to show that it is rather unspecialized, and, as such, is an excellent type for the study of evolutionary problems. It is fairly certain that the deciduous habit is derivative, and that types like *Hypoxis rooperi* are derived from evergreen types. The problem which suggests itself is how the deciduous type arose, and whether such an advance is difficult to attain. The figures given in Tables II and III seem to indicate that the deciduous habit, as shown by *Hypoxis rooperi*, is not difficult to establish. The analysis of *Hypoxis rooperi* leaves in autumn show that the death of the leaves does not bring about any great changes, and it appears that the leaves in autumn gradually become inert, and, finally, this inactivity brings about the death of the

leaves. With regard to the corms the carbohydrate, particularly the sugar, content grades slowly from the autumn to the winter condition, and the analysis of corms on May 1, when plants were still green but nevertheless showing signs of the approaching winter condition, shows that the plants have already approached the resting condition. The changes in both leaves and corms are so gradual that they might easily have occurred in an evergreen type, which passed through an inactive, although not leafless, condition. From such an evergreen type *Hypoxis rooperi* was probably derived, and the problems of ecological evolution would be simplified if the doctrine that change of function precedes change of form was as generally accepted as it ought to be. The ideas expressed in this paragraph are supported by the morphological changes in *Hypoxis rooperi* leaves. Here, again, one sees that the approach of the resting condition is gradual. The leaves on a corm do not all die together, and the leaves themselves may be dry towards the tips but still alive towards the base, especially at the colourless basal region.

Hypoxis rooperi Corms—Changes with the Age of the Plant.

It is well known that the storage regions of geophytes may become very large as the plants grow old. To test whether this increase in size is associated with any change of carbohydrate composition, a sample of six large corms, weighing 275, 325, 250, 230, 230, and 215 grm. respectively, was analysed and then compared with a sample of younger corms such as were used in the experiments on seasonal variations.

TABLE IV.

Carbohydrate Content of Young and Old Corms on May 1.
(Analyses Expressed as Percentages of Dry Combustible Material.)

	Young Corms.	Old Corms.
Water content . .	59.7	58.8
Total carbohydrates .	59.0	58.8
Total polysaccharides	54.4	53.7
Total soluble sugars .	4.6	5.1
Pentosans	3.25	3.20
Hexosans	51.1	50.5
Disaccharides . .	2.7	2.9
Monosaccharides .	1.9	2.2

Table IV shows the remarkable similarity in composition of young and old corms.

The way in which most geophytes produce larger and larger storage organs as they grow old is rather remarkable. No great changes in chemical composition or general appearance take place. What is the use of these large storage organs? The output of spring leaves does not keep pace with the increased size of the storage organs. Very young *Hypoxis rooperi* plants

have comparatively few leaves, but medium-sized corms produce just as many as old corms. The plants become mature at an early date. In many species of *Eriospermum* only one small leaf is produced annually, even on relatively enormous storage organs.

If we assume that the gross annual increase of plant material is proportional to leaf area, and that the rate of respiration is constant, then growth in *Hypoxis* obeys a simple interest law from an early stage, while in *Eriospermum* a simple interest law is obeyed practically throughout life. It is possible that some very interesting evolutionary series could be drawn from evergreen types, in which growth, while not necessarily obeying the compound interest law, certainly does not obey the simple interest law, to types like *Eriospermum*, where growth seems to obey the simple interest law very closely. *Hypoxis* would occupy an intermediate position in such a series.

PORTULACARIA AFRA LEAVES.

Before giving an account of carbohydrate changes, it is necessary to give an introductory discussion on the water relationships of succulent leaves. Succulents are characterized by the fact that they store large quantities of water and use some of this water during times of stress. Great changes in water content are therefore to be expected, and it is only reasonable to expect that any carbohydrates which are connected with the water relationship of the plant would also show great changes. Water content is generally expressed as a percentage of fresh weight. But it must be clearly understood that changes in water content relative to fresh weight give a very vague conception of changes in *actual*, not relative, water content of succulent tissues. We may illustrate this as follows. If the water content of a leaf, relative to fresh weight, rose from 80 per cent. to 90 per cent., the actual increase of water present, postulating a constant dry weight of, say, 10 grm., would be from 40 grm. to 90 grm., an increase of 50 grm., or 125 per cent. In other words, an increase in relative water content of 10 per cent. can mean an increase in actual water content of 125 per cent., presuming the amount of dry matter in the leaf remains constant. Similarly, a decrease in relative water content from 90 per cent. to 80 per cent., postulating a constant dry weight of, say, 10 grm., would mean a decrease from 90 grm. to 40 grm., or 55.5 per cent. in actual water content. Smaller decreases are generally found, but here the same differences between changes in relative and actual water content are found. A decrease in percentage water content from 86 per cent. to 85 per cent. means a decrease of 7.8 per cent. in actual water content, presuming the amount of dry matter remains constant. Obviously the higher the water content of a leaf, that is, the more succulent it is, the greater are the changes in actual water content for a given change in relative water content. This fact helps one to understand why the carbohydrate content of succulents changes so readily with

small changes in water content, expressed as percentages of fresh weight. It also explains why succulent leaves may have a very shrivelled appearance in spite of a fairly high percentage of water. Incidentally it explains why Knight (12) found that leaves whose percentage water content had been lowered by about 1 per cent. were definitely flaccid. Knight deduced from this fact that the cell-wall is only very slightly distended in the normal turgid state of the cell, but his argument loses some of its force if one realizes that a decrease in percentage water content from 86 per cent. to 85 per cent. (approximately Knight's figures) means a decrease of 7·8 per cent. in actual water content, as has already been shown.

Succulents undergo great variations in water content, and are influenced by very slight showers of rain. MacDougal and Spalding (14) have recorded this fact for *Carnegiea gigantea* and *Opuntia* sp. It seemed desirable to get some quantitative figures, and *Portulacaria afra* leaves are ideal for this purpose. A few light showers fell on the afternoon and evening of August 8. The amount of rain was so small that the water content of the soil at a depth of 8 in. was not changed. To test the effects of this rain, a sample of forty leaves were picked at noon on successive days, starting on August 8, when the weather was threatening, but the rain had not started falling. This sample was weighed, dried, and the dry weight found. From this the average fresh weight of a single leaf, the average amount of water in a single leaf, and the relative water content of the leaves could be found.

These are given in Table V.

TABLE V.

Increase of Actual and Relative Water Content of a Single (Average) Leaf after a Light Shower of Rain.

Time after rain in days.	Fresh weight of one leaf in grm.	Water content of one leaf in grm.	Increase in water content of leaves.	Water content expressed per cent. fresh weight.
0	0·2192	0·1810	—	82·6
1	0·2268	0·1902	5·1 %	83·9
2	0·2996	0·2594	43·3 %	86·6
3	0·2832	0·2462	36·0 %	86·9
5	0·3281	0·2896	60·0 %	88·3
7	0·3518	0·3113	72·0 %	88·5

The greatest variation found between several samples of forty leaves picked at the same time was 10·5 per cent. and 10·8 per cent. for fresh weight and water content respectively and 0·3 per cent. for percentage water content.

The figures in Table V show how quickly a succulent may absorb water, even after very light showers. Since the leaves were wet for a short period only, the bulk of the water must have been absorbed from the soil.

The roots of succulents are generally shallow. Cannon (15) has

stressed this fact for South African succulents, and *Portulacaria* seems to be no exception. This explains how they utilize very light rains, which do not wet the soil for more than a few inches. Light rains are almost as good as heavy rains. The figures given in Table V show that the light rains brought the leaves almost to normal turgidity, since the leaves of *Portulacaria afra* rarely contain more than 90 per cent. water. There is another fact to be considered. Veihmeyer (19) refers to the work of Alway and Rotmistrov, who independently come to the conclusion that water which has sunk to a depth of about 1 foot rarely reaches the surface except through the agency of a plant. Water which has penetrated the soil deeply is wasted as far as shallow-rooted plants are concerned, so that heavy rains are no better than moderate or light rains.

While shallow-rooted plants are fortunate in being able to utilize these rather common light rains, they are at a serious disadvantage in other respects. The surface layers of the soil dry rapidly, and although the suction pressure of the plant cells may be capable of absorbing any available soil water, the plant has difficulty in obtaining water because its movement by capillarity in dry soils is extremely slow. It is 'like trying to draw water from a dry well'. It follows that shallow-rooted plants are in an unhappy position during droughts when the surface of the soil is dry. Succulence is an ideal adaptation to such droughts because the water accumulated during a rain is used to help the plant over the lean period until another, not necessarily heavy, rain relieves the situation. There is a limit to the length of the adverse seasons which succulents can successfully stand, and this, together with the fact that light rains are of almost equal value to succulents as heavy rains, brings one to the conclusion that for succulents the distribution and not the total amount of rainfall is of prime importance. Indirect evidence for this statement is found in the distribution of succulents. In the Mediterranean type of climate, with wet winters and very dry summers, succulents are not common and do not thrive well, although the total annual rainfall is high. During summer the surface of the soil is dry, and succulents, whose roots cannot reach the water-table, are faced with very adverse conditions, combined with competition with the more fortunate, deep-rooted, often ericoid, types which dominate these regions. Incidentally the deep-rooted types, which extend their roots to the water-table and do not wait for water to reach them by capillarity, depend on sufficient rainfall to maintain the presence of a water-table and, for them, the total amount, and not the seasonal distribution, of rainfall is important. In dry regions, where the rainfall is fairly well distributed, even if small in amount, succulents are common. The south-western deserts of North America, which have two rainy seasons, have been called 'arboreal deserts'; the greenest of all deserts', and they abound in succulents.

THE SEASONAL VARIATION IN CARBOHYDRATE CONTENT OF
PORTULACARIA AFRA LEAVES.

The period under consideration extended from autumn, when conditions were not very dry, through the arid winter to spring, when rains brought the drought to an end. Carbohydrate analyses are expressed in one table as percentages of dry weight, and in the second as percentages of fresh weight. The differences between the two tables are considerable, for reasons already given, and the fresh weight is obviously the more natural. The changing amounts of the various classes shown in the fresh-weight table will represent very approximately the changing amounts to a given weight of water, and, for the classes concerned in the maintenance of the water relationships of the plant, demonstrates the value of fresh-weight tables when dealing with succulents and other types where the relationship between dry weight and actual water content varies considerably.

TABLE VI.

Seasonal Changes in Carbohydrate Content of Portulacaria afra Leaves.

(Analyses Expressed as Percentages of Dry Material.)

	May 9.	June 19.	July 30.	Aug. 17.	Oct. 3.
Water content . . .	87.0	82.6	78.3	85.4	87.6
Total carbohydrates . .	14.2	15.6	16.7	14.5	11.5
Total polysaccharides	13.0	14.9	16.1	13.6	10.2
Total soluble sugars . .	1.23	0.73	0.64	0.85	1.26
Pentosans	4.60	5.73	6.40	4.95	5.15
Hexosans	8.44	9.14	9.72	8.60	5.04
Disaccharides	0.82	0.34	0.26	0.31	0.38
Monosaccharides . . .	0.41	0.39	0.38	0.54	0.88
Water content of soil	18.6 %	16.3 %	14.9 %	13.8 %	23.1 %
Soil temperature . . .	18.6° C.	16.8° C.	16.8° C.	20.0° C.	19.1° C.
Remarks		dry.	very dry.		

TABLE VII.

Seasonal Changes in Carbohydrate Content of Portulacaria afra Leaves.

(Analyses Expressed as Percentages of Fresh Weight.)

	May 9.	June 19.	July 30.	Aug. 17.	Oct. 3
Water content . . .	87.0	82.6	78.3	85.4	87.5
Total carbohydrates . .	1.86	2.72	3.63	2.11	1.43
Total polysaccharides	1.70	2.59	3.50	1.98	1.27
Total soluble sugars . .	0.161	0.127	0.139	0.132	0.158
Pentosans	0.598	0.997	1.39	0.723	0.644
Hexosans	1.10	1.59	2.11	1.26	0.630
Disaccharides	0.107	0.059	0.055	0.053	0.048
Monosaccharides . . .	0.054	0.068	0.084	0.079	0.110

The analyses on June 19 and July 30 show the effects of drought. Pentosans and hexosans are high in amount, particularly in the fresh-weight tables. The unexpectedly high amount of pentosan as compared with hexosan on October 3 may possibly be due to the unavoidable inclusion of new spring leaves which, externally, were much the same as the old leaves. Judging from the dry-weight tables the soluble sugars tend to decrease with aridity, but this is not so clear in the fresh-weight tables, which show that disaccharides decrease regularly from autumn to spring, while the monosaccharides show a simultaneous increase. The reason is obscure.

The hexosans in the leaf of *Portulacaria afra* before photosynthesis commences are largely, or totally, colloidal. The iodine test for starch grains gives a negative result. Further evidence that the hexosans are not ergastic, storage products is furnished by a study of the diurnal changes in carbohydrate content of the leaves.

TABLE VIII.

Diurnal Changes in the Carbohydrate Content of Portulacaria afra Leaves.

(Analyses Expressed as Percentages of Dry Weight.)

	May 9. 6.30 a.m.	May 9. 5 p.m.	May 10. 6.30 a.m.
Water content . . .	87.0	86.2	86.8
Total carbohydrates .	14.2	20.8	14.5
Total polysaccharides	13.0	19.4	13.3
Total soluble sugars .	1.23	1.40	1.19
Pentosans	4.60	4.93	4.69
Hexosans	8.44	14.5	8.61
Disaccharides . . .	0.82	0.81	0.81
Monosaccharides . .	0.41	0.59	0.39

The first point to notice is that hexosans, the chief products of photosynthesis, increase largely during the day and decrease at night, so that the content on two consecutive mornings is fairly similar. This shows that most, if not all, the products of photosynthesis are translocated or respired. The great percentage increase during the day, representing as it does only the difference between the total amount of photosynthesis and the amount translocated or respired, would hardly be possible in the leaves if these were storage regions. A comparison with similar changes in the joints of cacti is instructive. The figures for total carbohydrate given by Spoehr in Table XIX are 16.18 at 5 p.m., 19.40 at 7.30 a.m. and 20.45 at 5 p.m. These are percentages of dry material. No regular diurnal change is apparent in these organs, which contain storage material.

It has already been stated while discussing errors that the amount of hexosans and pentosans on two consecutive mornings may be different,

although, even when this difference is fairly high, it does not obscure the fact that hexosans increase during the day and decrease again at night. When pentosans increase, hexosans also increase, and vice versa. Except for changes due to photosynthesis, all changes in pentosans were accompanied by similar changes in hexosans. Seasonal changes, and the changes due to leaves being on different sides of the tree (see Table IX), illustrate this. Before sunrise no starch grains are present. All this evidence seems to point to the fact that the hexosans present before photosynthesis resemble the pentosans and have a similar colloidal nature. It seems highly desirable that further work should decide whether this conclusion is tenable. If colloidal hexosans behave like pentosans and increase with aridity it would tend to show that it is the physical (colloidal) and not the chemical properties of pentosans which cause them to increase with low-water content. Various workers regarded pentosans as inert and perhaps waste products. The increase of pentosans during periods of stress, when metabolism is probably at a low ebb, might be due to their chemical inertness, although this seems unlikely. Any proof that colloidal hexosans and pentosans behave similarly with regard to aridity would show that carbohydrate colloids, in general, increase with low-water content.

The increase of pentosans during the day might be explained either by assuming that pentosans are produced during photosynthesis or by assuming that the fall in water content increases the amount of pentosans.

Differences between Leaves from Opposite Sides of the Tree.

While experimenting to eliminate differences between samples of leaves we stumbled across the fact that leaves picked from the north side had a very different carbohydrate content from those from the less exposed south side. This difference was not only confined to carbohydrates, but a glance at fresh leaves or at the alcoholic extract of the leaf powders showed that the leaves on the north side were deficient in chlorophyll. Later on in the year, when spring rains had fallen, differences of colour were less marked or even absent. Light intensity is much greater on the north side, but the evaporation rates measured by atmometers did not differ much, a 6 per cent. difference for the day being recorded.

Residual pentosans not hydrolysed by the dilute acid in $4\frac{1}{2}$ hours formed 3.28 per cent. and 3.45 per cent. of the dry weight on the south and north sides respectively.

The figures in Tables IX and X show that the north side is much richer in pentosans and hexosans, especially if the fresh-weight table is studied. Sugars are richer on the south side, but the fresh-weight tables show that this difference is relatively small.

These facts are interesting in many ways.

TABLE IX.

The Carbohydrate Contents of Leaves from the North and South Sides of the Same Tree on May 9.

(Analyses Expressed as Percentages of Dry Material.)

	South.	North.	Difference per cent.
Water content	88.6	87.0	—
Total carbohydrates . .	11.6	14.2	22.4
Total polysaccharides .	10.1	13.0	28.7
Total soluble sugars . .	1.48	1.23	18.0
Pentosans	3.50	4.60	31.4
Hexosans	6.63	8.40	26.7
Disaccharides	0.95	0.82	13.7
Monosaccharides . . .	0.53	0.41	22.5

TABLE X.

(The Figures given in Table IX are here Calculated as Percentages of Fresh Weight.)

	South.	North.	Difference per cent.
Water content	88.6	87.0	—
Total carbohydrates . .	1.33	1.86	39.8
Total polysaccharides .	1.16	1.70	46.6
Total soluble sugars . .	0.171	0.161	5.8
Pentosans	0.399	0.598	49.9
Hexosans	0.756	1.10	45.5
Disaccharides	0.111	0.107	3.6
Monosaccharides . . .	0.060	0.054	10.0

(1) As would be expected, the carbohydrate content of the sunny, north side shows a xerophytic tendency.

(2) Great care must be exercised in selecting samples for analysis.

(3) A plant is not a single physiological unit, but is plastic and adaptable.

(4) The figures are interesting from the standpoint of ecological evolution and plant succession.

Portulacaria afra is a pioneer tree in plant succession and, as such, is subjected to varying conditions. Physiological plasticity is obviously desirable. While this species appears early in plant succession, it is phylogenetically an advanced, derivative type, which is generally the case with pioneer species. Reference has already been made to the theory that the central, primitive types of angiosperms were moist tropical trees. Derivative sub-tropical types left the moist tropical forest and invaded drier regions, and this could only take place provided the invaders were plastic and equally at home in the forest or forest margin and in the derivative

habitat. Bews and Aitken (4, section 5) have given an account of the remarkable plasticity of *Cussonia spicata*, which may act as a pioneer in tree-veld succession or may occur in close forest, and this indicates that great plasticity is possible. The figures in Tables IX and X go to confirm that the plasticity required by this theory is not difficult to postulate. In invading drier regions the pioneer types changed their functional activities, and changes of form would probably follow. Bews and Aitken (4, section 1) found that the size of the aeration systems of leaves, even from the same tree, is very variable, and the species which appear early in plant succession have a greater degree of variability in their physiological structure and functions than those less specialized types which appear later.

(5) The great physiological variability shown by Tables IX and X, and by the fact that different carbohydrate contents are encountered on consecutive days—a fact we have already mentioned when discussing errors—shows how easily succulents adapt themselves to environmental conditions. These changing adaptations contrast with the fixed morphological adaptations, such as spinosity, and lignification of leaves, which are common among xerophytes. The plastic adaptations have an advantage over fixed adaptations to aridity in that they enable a plant to become less xerophytic when environmental conditions are more favourable, but we know little about the relative efficiency of plastic physiological and fixed morphological adaptations in withstanding drought. The great advantage in having plastic adaptations is obvious, and experimental work is required in regard to the relative efficiency of physiological responses to aridity.

SEASONAL VARIATIONS IN CARBOHYDRATE CONTENT OF *PORTULACARIA AFRA* TWIGS.

Samples of twigs were collected from the autumn, through the arid winter, to the spring. The tree produced new leaves in great numbers during September and early October, so the effect of renewed vegetative growth on the storage regions would be detected from the analyses of the twigs, which are packed with starch. A few small flowers were produced in October, but they are hardly worth considering because they were so small in size and number.

Residual pentosans varied, within small limits, about 3.8 per cent. dry weight.

Changes in sugar content are again difficult to explain. There is a general tendency to lower the sugar content in winter. Disaccharides increase steadily from autumn to spring just as disaccharides decrease and monosaccharides increase steadily from autumn to spring in the leaves. This tempts one to regard cane sugar as the chief translocation form in autumn and monosaccharides as the chief translocation form in spring. But there are insufficient facts to draw any definite conclusions, and in any

case the problem of translocation of photosynthetic products by small concentrations of sugars is difficult to understand.

TABLE XI.

Seasonal Changes in Carbohydrate Content of Portulacaria afra Twigs.

(Analyses Expressed as Percentages of Dry Weight.)

	May 18.	June 19.	July 30.	Aug. 17.	Oct. 3.
Water content . . .	74.3	71.4	68.6	71.3	71.9
Total carbohydrates . . .	17.6	21.3	19.0	19.3	20.2
Total polysaccharides . . .	16.5	20.6	18.3	18.4	19.3
Total soluble sugars . . .	1.14	0.74	0.67	0.94	0.94
Pentosans	4.14	4.28	4.70	4.24	4.47
Hexosans	12.32	16.32	13.6	14.2	14.8
Disaccharides	0.22	0.26	0.36	0.42	0.41
Monosaccharides	0.92	0.48	0.31	0.52	0.53
Water content of soil . . .	19.9%	16.3%	14.9%	13.8%	23.1%
Soil temperature	17.2° C.	16.8° C.	16.8° C.	20.0° C.	19.1° C.
Remarks		dry	very dry		new leaves produced

Pentosans increase slightly with decreasing water content, but the October value is rather high. It is remarkable that in the three perennial tissues analysed—*Hypoxis rooperi* corms, *Portulacaria afra* leaves and twigs—the spring values are higher than one would expect if pentosans decreased with increasing water content. Is this to be correlated with a greater water absorbing capacity in spring?

The twigs are packed with starch, so some of the hexosans are storage substances. Hexosans do not seem to be depleted by renewed vegetative growth in spring. Since only twigs were analysed, it is possible that the high hexosan content in spring was due to carbohydrates being moved up from the larger branches to replace any carbohydrates lost by the young leaves. Curtis (6), however, concludes from a long series of experiments that it is very probable that there is normally no upward movement of foods from the roots and perhaps little, or none, from the main trunk. Another possibility is that the twigs recovered some carbohydrate from the old leaves, which showed a decreased carbohydrate content in spring. But the amount involved is less than a day's photosynthetic increase. Judging from microchemical examinations, we think that similar facts hold for succulent *Crassulas*, whose stems possess much starch even after fruiting in mid-winter and after spring growth.

CONCLUSIONS.

The starch stored in the species studied did not seem to be used to any appreciable extent during periods of renewed growth, although the season

was probably more severe than usual. Is a reserve of starch needed for the use of young growing material? It is quite probable that young leaves become self-supporting, as far as carbohydrates are concerned, at a very early stage, and the fact that only a small part of the food reserves of a seed may be used during germination shows us that one should not assume *a priori* that food reserves are always used. Moore (15), dealing with deciduous trees, was also led to wonder to what extent storage is necessary for leaf growth, although he was impressed by the completely different fact that many trees have practically no storage reserves.

In view of the importance attached to the storage of starch and the fact that it is a fairly reliable index to evolutionary advance, the whole question needs to be studied. Is the storage of starch always an adaptation or must we break away from a purely teleological view and adopt other ideas? Hartwell's work (22), to which we were only able to refer in an abstract, favours the latter course. He found that starch congestion was brought about in the above-ground portion of the potato plant by conditions which cause a retardation of growth. These conditions do not seem to interfere with photosynthesis. We may well inquire whether the storage reserves in some of our slow growing plants are adaptations or simply the dumping ground of carbohydrates which are produced in excess of requirements, and whether the lack of starch in our hard-wooded tropical trees is because no storage reserves are required, or because no excess carbohydrate is present to accumulate. No answer can be given as yet, and both sides are probably partly true.

Very little can be said with regard to the sugars. They do not seem to be directly affected by water content because very regular decreases or increases may occur during totally different fluctuations of water content.

We wish to deal fairly fully with the question of colloidal carbohydrates because there has been so much interest attached to this subject lately.

Spoehr found that aridity and high temperatures caused an increase of pentosans in cacti. Our experiments on succulents showed a similar increase with aridity and low temperatures. Aridity would therefore seem to be the chief factor influencing pentosan content. For reasons which have been given, it seems probable that colloidal hexosans behave similarly to colloidal pentosans.

There is a natural tendency to associate these changes with succulents alone, but this may not be correct. If increases in pentosan content are due to decreases of water content then great increases of pentosan content would be expected in succulents only because here one finds great changes in water contents that are not met elsewhere. Knight (12) has shown that ordinary mesophytic leaves are incapable of great changes in relative water content, and this is probably more or less true for most leaves except

succulent leaves. The actual meaning of the changes of relative water content in succulents has already been stressed. If changes of pentosan content are due to changes in water content, one would expect the increases or decreases of pentosans in non-succulent types would be small. Inconclusive evidence indicates that possibly small changes in pentosan content are found in non-succulent types, e. g. *Hypoxis*, *Portulacaria* twigs. Doyle and Clinch's (8) experiments on conifer leaves might point this way. Farnell (10) found that the pentosan content of cane-juices increased with the interval between cutting and milling, a result, he states, which was first observed by Steuerwald. It is quite probable, therefore, that non-succulent types behave like succulents in this respect but to a lesser degree.

In passing it might be mentioned that pentosans do not always increase with decreasing water content, for occasionally decreases in water content are accompanied by decreases in pentosan content. An example is afforded by young leaves which may have high pentosan and relative water contents, but both decrease at maturity. In young cells a relatively large amount of the water content of the cell is accommodated by colloids, but later on proportionally less and less colloids occur in the cell.

These various interesting relationships between colloids and water content deserve more attention, and it would be interesting to know how much of the high pentosan content in dry seasons is due to colloids occupying a greater portion of the cell space, i. e. how much more water is accommodated by colloids during drought, and how much is due to the colloids of the cell getting a greater proportional dry weight and a greater imbibition force.

A discussion on the imbibition forces of plant colloids from the quantitative point of view is interesting, and fortunately there are sufficient facts to enable one to discuss the subject. Colloids may have large or small imbibition forces according to the degree to which they are hydrated, and we will show that, for succulents particularly, the plant colloids may have a small imbibition force.

Within a plant water is held both by colloids and in vacuoles. It is obvious that a state of equilibrium must exist between these two water-holding mechanisms when one is not absorbing from the other, and it is only reasonable to postulate that there will always be a tendency to establish and maintain this equilibrium although it might be disturbed during transpiration. If, for lack of better terms, we define the 'suction pressure' of a colloid in, say, a gum canal as the difference between the imbibition force of the colloid and the 'turgor pressure' of the gum canal walls, &c., on the colloid, then within any tissue the 'suction pressure' of the colloids in the canals equals the suction pressures (used in the ordinary sense) of the cells when the equilibrium postulated above has been established.

This seems to be the only reasonable way of approaching the subject

in a tissue with, say, gum canals or purely mucilaginous cells, but one can leave out suction pressures when considering the relationship between protoplasmic colloids and cell vacuole within the cell. The inner plasmatic membrane defining the vacuole from the cytoplasm is not a rigid structure, and the turgor pressure of the cell wall would effect to an equal degree all parts of the fluid protoplast. When the cell is in a state of equilibrium the protoplasmic colloids are neither losing to, nor gaining from, the vacuole, and the imbibition forces of the protoplasmic colloids cannot be greater than the net osmotic pressure of the vacuole.

The osmotic pressures of succulents are exceptionally low for xerophytes, and contrast with the high osmotic pressures of the sclerophyllous xerophytes among which they grow. The fact has long been established by many workers. From the preceding paragraph it will be seen that the colloids within the cells of succulents must have similarly low forces of imbibition. Several writers seem to have been mistaken on this point.

The significance of low osmotic and imbibition pressures in succulents is a difficult problem, although the subject is simplified if we recognize that the water-storage tissues of succulents have a dual function. Not only must they absorb water, but they must be able to lose it to the less specialized tissues during desiccation. The low osmotic pressures of these water-storage tissues may be an adaptation to allow these tissues to carry out both these functions.

When dealing with the absorption of water by plants one must consider suction, rather than osmotic, pressures. A low osmotic pressure can be compensated for by a low turgor pressure, and this is probably important when considering succulents. Succulents may lose a lot of water and the tissues become extremely desiccated. When rain falls the gain of water by these tissues is very large (see Table V), and they swell considerably. We must, therefore, assume that, in their desiccated state, the cells of the water storage tissues have a very low turgor pressure, and their suction pressure is relatively high and efficient. When succulents are affected by drought their low osmotic pressures are not particularly disadvantageous because of their relatively high suction pressures. When rain falls their turgidity is partly restored, and the suction pressure falls and becomes less efficient, so that finally no further absorption of water takes place. In all plants, whatever their osmotic pressures, such a state must be reached, and this may partly account for the presence of a 'water deficit' in leaves even during favourable conditions.

Another aspect of the subject is interesting. Under dry conditions, when the soil solution is more concentrated and water is difficult to obtain, a shallow-rooted plant could not obtain much water even if its osmotic, or suction, pressure were high. The slow movement of water by capillarity in the dry surface layers of the soil, and not the suction pressure of the plant,

is possibly the limiting factor in water absorption. In this respect the low osmotic pressures of shallow-rooted succulents may not be a disadvantage. Still other aspects of the subject are interesting. When a succulent organ loses water during times of stress the whole organ decreases in volume. This may be correlated with the thin collapsible walls often possessed by succulent tissues, and, in turn, the thin walls might be correlated with the low osmotic pressures of succulents, since it is hardly probable that thin walls could withstand the enormous stresses imposed on the plant by a high osmotic pressure during times of plenty.

Another point worth considering is that during lean periods, when the leaves are far from turgid, the suction pressure of a succulent must be almost equal to its osmotic pressure. A high suction pressure would arise if succulent tissues had high osmotic pressures, and would scarcely be desirable if continued for the great stretches of time during which succulents show signs of desiccation. Knight (12) has suggested that the water columns of a plant may be ruptured by the increased tension during wilting, and it seems probable that in succulents the low osmotic pressure is helpful in reducing the tension to a minimum.

The imbibition forces of water-storing plant colloids have often been overestimated. Statements that mucilaginous cells and tissues 'can act as water reservoirs, owing to the fact that the more or less viscid mucilage which they contain has a *stronger* attraction for water than ordinary cell sap', are misleading and contain two conflicting ideas. When an organ loses water, both the water storage and other tissues lose a certain amount of their turgidity, and acquire greater powers of attracting water, i. e. suction pressures. This increased suction pressure possessed by the water-storing tissues must be exactly equal to that possessed by other tissues as soon as an equilibrium is reached. If, therefore, the water-storing tissues have greater osmotic or imbibition pressures than have the other tissues they must have the greater turgor pressures to maintain the equilibrium between the various suction pressures. But water-storing tissues, because of their function, lose the most water and have, therefore, the lowest turgor pressures. The ideas quoted above are not, therefore, tenable. The case is clearly shown by the gum in the canals in *Hypoxis rooperi* corms. This gum is under great pressure, since, on cutting a corm in half, it oozes out *immediately*, and has, therefore, a greater attraction for water than the rest of the corm, but is kept from absorbing more water by its high 'turgor pressure'. To keep a relatively high 'turgor pressure' the gum can never lose much water, and is consequently useless as a water-storage system.

Since the colloids within a plant, whether they are extra-cellular or intra-cellular, must always have a water-attracting force in equilibrium with the vacuoles, it appears that the study of cell suction pressure is sufficient to cover the net forces of water attraction of all the hygroscopic systems

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Another aspect of the subject is interesting. Under dry conditions, when the soil solution is more concentrated and water is difficult to obtain, a shallow-rooted plant could not obtain much water even if its osmotic, or suction, pressure were high. The slow movement of water by capillarity in the dry surface layers of the soil, and not the suction pressure of the plant,

is possibly the limiting factor in water absorption. In this respect the low osmotic pressures of shallow-rooted succulents may not be a disadvantage. Still other aspects of the subject are interesting. When a succulent organ loses water during times of stress the whole organ decreases in volume. This may be correlated with the thin collapsible walls often possessed by succulent tissues, and, in turn, the thin walls might be correlated with the low osmotic pressures of succulents, since it is hardly probable that thin walls could withstand the enormous stresses imposed on the plant by a high osmotic pressure during times of plenty.

Another point worth considering is that during lean periods, when the leaves are far from turgid, the suction pressure of a succulent must be almost equal to its osmotic pressure. A high suction pressure would arise if succulent tissues had high osmotic pressures, and would scarcely be desirable if continued for the great stretches of time during which succulents show signs of desiccation. Knight (12) has suggested that the water columns of a plant may be ruptured by the increased tension during wilting, and it seems probable that in succulents the low osmotic pressure is helpful in reducing the tension to a minimum.

The imbibition forces of water-storing plant colloids have often been overestimated. Statements that mucilaginous cells and tissues 'can act as water *reservoirs*, owing to the fact that the more or less viscid mucilage which they contain has a *stronger* attraction for water than ordinary cell sap', are misleading and contain two conflicting ideas. When an organ loses water, both the water storage and other tissues lose a certain amount of their turgidity, and acquire greater powers of attracting water, i. e. suction pressures. This increased suction pressure possessed by the water-storing tissues must be exactly equal to that possessed by other tissues as soon as an equilibrium is reached. If, therefore, the water-storing tissues have greater osmotic or imbibition pressures than have the other tissues they must have the greater turgor pressures to maintain the equilibrium between the various suction pressures. But water-storing tissues, because of their function, lose the most water and have, therefore, the lowest turgor pressures. The ideas quoted above are not, therefore, tenable. The case is clearly shown by the gum in the canals in *Hypoxis rooperi* corms. This gum is under great pressure, since, on cutting a corm in half, it oozes out *immediately*, and has, therefore, a greater attraction for water than the rest of the corm, but is kept from absorbing more water by its high 'turgor pressure'. To keep a relatively high 'turgor pressure' the gum can never lose much water, and is consequently useless as a water-storage system.

Since the colloids within a plant, whether they are extra-cellular or intra-cellular, must always have a water-attracting force in equilibrium with the vacuoles, it appears that the study of cell suction pressure is sufficient to cover the net forces of water attraction of all the hygroscopic systems

within a plant, provided the general equilibrium has not been disturbed. To obtain a complete insight into the value of the changes of colloidal content with increasing or decreasing aridity, one must consider other properties of colloids as well as their imbibition pressures.

With regard to the possibility that colloids may check transpiration, one must remember that the evaporating power of the atmosphere is very high (according to Shull (17), it averages about 1,000 atmospheres). No plant is likely to develop forces to oppose this to any appreciable extent. The only practical way for a plant colloid to check evaporation from its surface is to expose a small free-water surface, i.e. to be only slightly hydrated, and so to form a sort of cuticle. How far this occurs in the colloids lining air spaces is difficult to say.

In conclusion we might emphasize that the study of the value of plant colloids should proceed along many lines which have been more or less neglected. Their resistance to the movement of water and, perhaps, solutes, the advantages or disadvantages of a greater portion of cell space being occupied by mucilaginous colloids, and other problems need urgent investigation.

SUMMARY.

1. Monosaccharides and disaccharides were extracted with aqueous alcohol and their respective reducing powers estimated before and after inversion under Herzfeld conditions. Polysaccharides were estimated after hydrolysis with 1.1 per cent. hydrochloric acid for $4\frac{1}{4}$ hours on a boiling water-bath. The reducing powers of sugars were estimated by means of excess Fehling's solution, and the excess was determined iodimetrically or by a new, quick modification of the titanous chloride method. Pentosans were estimated by the Kröber method, the pentoses produced by the hydrolysis of the pentosans being separated from unhydrolysed pentosans by means of aqueous alcohol.

2. The evolution of the deciduous habit in geophytes does not appear to be a difficult accomplishment.

3. Figures are given for the response of succulent leaves to light showers of rain. There are reasons for thinking that, for shallow-rooted succulents, the distribution of rain throughout the year is more important than the annual total amount.

4. In tissues with a high-water content changes in actual water content are very different from changes in relative water content.

5. In *Portulacaria afra* pentosans, and possibly mucilaginous hexosans, increase with lowered water content. The significance is far from clear.

6. Some relationships between the vacuolar and colloidal systems in plants are given. In succulents the force with which the intra-cellular colloids attract water is not great.

7. The composition of *Portulacaria afra* leaves varies greatly with the side of the tree on which they occur. The significance of this fact is discussed.

8. In the plants studied the large carbohydrate reserves are drawn upon to only a very slight extent when new leaves are produced in spring. Its possible bearing on current ideas on carbohydrate storage are mentioned.

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An Investigation into the Bacterial Associations of Some Cyanophyceae, with Especial Reference to their Nitrogen Supply.

BY

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INTRODUCTION.

IT is a matter of frequent observation that members of the Cyanophyceae grow prolifically in regions, like sandy wastes, which are presumably lacking in the ordinary plant food-materials, since other, and higher plants, do not obtain a footing there. The problem of the nitrogen supply is especially urgent, and has given rise to the suggestion by Heinze and others that members of the Cyanophyceae are able to assimilate atmospheric nitrogen. Other workers, including Kossowitsch, Bouilhac, and Fischer, deny this, and ascribe the fixation of atmospheric nitrogen to the activity of nitrogen-fixing bacteria. Literature on the question is scanty, and many of the methods employed by workers appear crude in the light of modern technique.

In 1889, whilst experimenting on the growth of fern prothalli in culture media, Prantl (9) observed the presence of species of *Anabaena* and *Nostoc* in culture solutions free from nitrogen. By special culture methods he obtained an extensive growth of these forms in media devoid of nitrogen. From the undoubted increase in growth he assumed an increase in the nitrogen content, and he further suggested that their ability to use free nitrogen threw light on the symbiotic relations between the Cyanophyceae and other plants, such as *Blasia*, *Azolla*, etc.

Little account can be taken of this work since there is no proof, by chemical analysis, of an increased nitrogen content; and, further, there was apparently no attempt to obtain pure algal cultures free from contaminating micro-organisms.

Kossowitsch (6) in 1894 investigated the question whether soil bacteria took any part in the nitrogen fixation which had been attributed to certain algae.

He had two series, one comprising eighteen flasks, each containing

a layer of sand and a suitable nutritive medium. Each flask was inoculated with pure *Cystococcus*. In a second series there were five pairs of flasks similarly arranged with sand and a nutritive medium which varied with the alga to be investigated. One of each pair had sugar added to the sand and the nutritive medium. In addition to the alga used in the first series two others were used in this series, namely, a species of *Nostoc* and *Stichococcus*. Soil bacteria were added to the algae in the second series.

In the first series there was no fixation of nitrogen, either with or without sugar. In the second series there was fixation in nearly every case. *Nostoc* with bacteria showed the greatest fixation of nitrogen. In the case of *Cystococcus* and bacteria there was scarcely any fixation without sugar, but there was considerable fixation with sugar: from this Kossowitsch concluded that it was not the *Cystococcus* but the bacteria which fixed the nitrogen. He further suggested that some algae, especially gelatinous ones, take the place of sugar in supplying the nutriment to the bacteria which live in the gelatinous substance.

A question which arises at once is why Kossowitsch found it necessary to add soil bacteria to his algal cultures when he states that the gelatinous substance of the algae is full of bacteria. Also, it is not clear whether his pure algal culture used in his first series was freed from these bacteria.

He used only two flasks per alga in his second series, and, taking into account the variations in growth, etc., which exist among both algae and bacteria, this experiment appears to furnish slight evidence upon which to base such a wide generalization.

Similar work was done two years later by Bouilhac (1), who inoculated six flasks of nutritive media free from nitrogen, with *Nostoc punctiforme* and soil bacteria. He estimated the nitrogen content by the Kjeldahl method and found an increase in nitrogen.

Here, again, insufficient data were obtained on which to base a sound conclusion. Bouilhac, too, has overlooked the possibility of the presence of nitrogen-fixing bacteria in the algal sheath.

In 1903 Bouilhac (2) did further work on this question, in conjunction with Giustiniani. In this case only four flasks were used, two of which acted as control flasks without any addition of alga. The other two flasks were inoculated with *Nostoc punctiforme* and a species of *Anabaena* with their bacteria. After six weeks there was a marked increase in the nitrogen content of the inoculated flasks, as compared with that of the control flasks.

As in his previous work, the results are too meagre to form any positive proof of nitrogen fixation by members of the Cyanophyceae in conjunction with bacteria.

Fischer (4) in 1904 obtained a species of *Azotobacter* in pure culture from colonies of a species of *Oscillatoria*. He was unable to identify the

Azotobacter with absolute accuracy, but he postulated a mutual exchange of carbohydrate and nitrogen compounds between the algae and the bacteria.

In 1906 Heinze (5) worked with cultures of organisms taken from fallow soils which contained a good cyanophycean vegetation. He turned his attention to *Nostoc*, which he found grew luxuriantly in nitrogen-free and sugar-free solutions containing lime. *Azotobacter* was not present in the culture, and possible fixation by bacteria or mould fungi was prevented by the absence of carbohydrate. Therefore, Heinze concluded, the work of nitrogen-assimilation must be ascribed to the *Nostoc* alone.

Apparently the amount of nitrogen in the inoculum was not ascertained, and this might easily amount to the so-called fixation in crude culture—that is, 2 to 3 mg.

It is not stated whether the experiments were carried out in daylight or not. If they were, then the carbohydrate material resulting from carbon assimilation would be available for bacterial growth. And even if they were in the dark, the carbohydrate material from the mucous investment of the algal wall is a possible source of food for bacteria.

There is no method indicated by which the *Azotobacter* was eliminated from the crude culture; and, finally, no account seems to have been taken of other nitrogen-fixing bacteria which may have been present.

In 1920 Moore and Webster (8) obtained an increase in the nitrogen content of a flask of water in which algae had been allowed to develop naturally. These algae were found to be unicellular members of the Chlorophyceae.

The methods employed by these workers seem very crude, and there appears to have been no attempt at sterilization, the absence of turbidity being taken as indicating an absence of bacterial growth. Very little importance can be attached to a conclusion based on a series consisting only of two flasks.

In 1921 Wann (10) claimed to have obtained an increase in the nitrogen content of seven species of Chlorophyceae grown in pure culture in media containing nitrate and glucose.

This work was repeated in 1923 by Bristol and Page (3). They obtained considerable and even luxuriant algal growth under similar conditions to those of Wann. But they were unable to obtain evidence of nitrogen fixation. They showed Wann's method of chemical analysis to be faulty and likely to yield figures giving the appearance of such fixation. Further, Wann's results can be regarded as inconclusive since it was only on media containing nitrate that he claimed to obtain definite fixation.

The available information on the bacterial associations of the Cyanophyceae is, therefore, so insufficient that it appeared profitable to make a more exhaustive examination of the nitrogen relations of some of the algae concerned.

PRELIMINARY EXAMINATION FOR THE BEST METHOD OF CULTURE
OF THE CYANOPHYCEAE UNDER INVESTIGATION.

The types of algae selected for investigation were:

Nostoc, sp.
Rivularia, sp.
Gloeocapsa, sp.

The material was carefully washed with distilled water to remove any extraneous matter present. In order to find the medium best suited to the growth of these algae four nutrient solutions were tested:

I. *Detmer-Moore's Nutrient Solution.*

KNO ₃	7 gm.
NaCl	1.5 gm.
K ₂ HPO ₄	1.5 gm.
MgSO ₄	1.5 gm.
CaSO ₄	5 gm.—in excess
Fe ₂ Cl ₆	a few drops

Made up to 3,000 c.c. with distilled water.

II. *Knop's Nutrient Solution.*

KNO ₃	1 gm.
K ₂ HPO ₄	1 gm.
MgSO ₄	1 gm.
Ca(NO ₃) ₂	3 gm.
Fe ₂ Cl ₆	a few drops

Made up to 6,000 c.c. with distilled water.

III. *Artificial Sea-water.—Allen.*

NaCl	28.13 gm.
KCl	0.77 gm.
CaCl ₂	1.20 gm.
MgCl ₂	2.55 gm.
NaHCO ₃	0.11 gm.
MgSO ₄	3.5 gm.

Distilled water—1 litre.

IV. *Natural Sea-water.*

Obtained direct from the sea as required and filtered before use.

The Detmer-Moore and Knop solutions were used at one-fifth of their normal strength.

Three series of flasks were started:

- (i) Erlenmeyer flasks with 10 c.c. of liquid medium only.
- (ii) Erlenmeyer flasks with sand saturated with 10 c.c. of medium.
- (iii) Erlenmeyer flasks with agar-agar medium, prepared by adding 2 per cent. of agar-agar to 10 c.c. of the nutrient solution under examination.

The growth of each of the three algae was tested in each of the four solutions under each of the three conditions stated above (i), (ii), (iii).

All flasks were sterilized in an autoclave, and, after cooling, each was inoculated with a small mass of the requisite alga and plugged with cotton wool, which had been flamed. The whole series was placed in a greenhouse, and at the end of two weeks each flask was examined, macroscopically and microscopically, to determine which medium had proved the best for promoting healthy growth. The most successful methods of culture for the algae investigated, based initially upon their macroscopic appearance, are indicated at the heads of the respective columns below, the other methods following in their order of success. This order was confirmed throughout by microscopic examination, based upon the size of the individual cells, their intensity of colour, and the length of the filaments.

Nostoc.

1. Detmer-Moore solution on sand.
2. Sea-water on sand.
3. Knop solution on sand.
4. Artificial sea-water on sand.
5. Artificial sea-water with agar-agar.
6. Artificial sea-water alone.
7. Sea-water with agar-agar.

Gloeocapsa.

1. Artificial sea-water on sand.
2. Artificial sea-water with agar-agar.
3. Sea-water on sand.
4. Knop solution on sand.

Rivularia.

1. Knop solution on sand.
2. Artificial sea-water on sand.
3. Detmer-Moore solution on sand.
4. Knop solution with agar-agar.
5. Detmer-Moore solution with agar-agar.
6. Sea-water on sand.
7. Sea-water with agar-agar.

An outstanding feature of this examination was the success of sand as a medium for growth. The only exception, that of *Gloeocapsa* with Detmer-Moore solution, was due to contamination by filamentous Chlorophyceae. Another feature which was outstanding was the relative unsuitability of a liquid medium, for even in the one case where this medium

was apparently successful, i.e. that of *Nostoc* in artificial sea-water, the alga could not be said to be in a flourishing state.

The nutritive requirements of the algae appeared to vary considerably with the genus under consideration. It was noted particularly that *Gloeocapsa* flourished in artificial sea-water, a medium without any nitrate, or combined nitrogen of any kind.

THE EXAMINATION OF THE NITROGEN-CONTENT OF ALGAL CULTURES GROWN ON SAND.

Having ascertained the artificial nutritive media most suited to the algae under consideration, it was decided to find out whether there was any increase in the nitrogen-content of these algae after they had been growing for some time in the artificial media selected.

To this end, flasks containing a specified quantity of solution and sand were sterilized in an autoclave, and weighed quantities of alga were added to each. The flasks were plugged with cotton wool and put in a greenhouse at a temperature as near to that of summer heat as could be regulated. Every few days the position of the flasks was changed, so that there was general equal distribution of illumination.

In order to ascertain the initial nitrogen content of each flask, control flasks, containing media only, were prepared, and to each was added 25 c.c. concentrated sulphuric acid. These flasks were put aside for estimation of their nitrogen-content later. Similarly, in order to estimate the initial nitrogen content of the alga put into each flask quantities of alga were weighed out and put into distilling flasks with 25 c.c. of concentrated sulphuric acid, and these also were left for subsequent estimation of their nitrogen-content.

The sand was washed, dried, and sieved, and the algal material was washed and freed from extraneous matter. There were four groups of flasks:

- I. *Rivularia* in Knop's nutrient solution.
- II. *Nostoc* in Detmer-Moore's nutrient solution.
- III. *Nostoc* in Knop's nutrient solution.
- IV. *Gloeocapsa* in artificial sea-water.

There were fifteen flasks in each group, twelve of which were to be inoculated with alga, and the other three to be left as controls for estimating the nitrogen-content of the medium.

30 grm. of dried sand was added to each flask, and to this was added, by means of a pipette, 10 c.c. of the requisite nutritive solution, the Knop and Detmer-Moore solutions being used at one-fifth of their normal strength.

The flasks, containing 30 grm. of sand and 10 c.c. of liquid, were sterilized in an autoclave, and after removal from the autoclave each flask was immediately plugged and a sand slant was formed by tilting the flasks, which were then left to cool.

For inoculation, the alga, having been thoroughly washed and left to drain in a filter, was weighed out in separate small quantities in tared, sterilized watch-glasses, and each separate amount was removed to its appropriate flask with a sterile needle immediately after weighing. At the same time separate larger amounts of each alga were weighed out and transferred to distilling flasks to which 25 c.c. concentrated sulphuric acid was added, and these were put aside for a later examination of the initial nitrogen-content of the algae concerned. The inoculated flasks were plugged immediately with flamed cotton wool and placed on a shelf in a greenhouse.

At the end of 182 days the series was removed from the greenhouse for the purpose of estimating the nitrogen content of the flasks. All the flasks showed signs of increased growth, although in some cases, especially in the *Gloeocapsa* group, this was apparently slight. Many of the *Nostoc* inoculations seemed to have lost their typical gelatinous nature and had adopted a 'spreading' habit. The *Rivularia* was in the most normal state, although in every case the alga was living and had retained its colour. Doubtless there was contamination by various forms of Chlorophyceae, but since it has been stated, and is generally accepted, that this group is unable to 'fix' atmospheric nitrogen [Bristol and Page (3)], this fact can be ignored for the purpose of this investigation.

Estimation of the Nitrogen Content.

The procedure adopted for each flask was the following slight modification of the Kjeldahl process:

The contents, i. e. sand, alga and liquid, were carefully transferred to a distilling flask of 700 c.c. capacity, with the minimum of distilled water, and 2 grm. of salicylic acid and 5 grm. of powdered zinc were added. 25 c.c. of concentrated sulphuric acid was then transferred to the Erlenmeyer flask, and, after rotation of the flask to ensure the action of the acid on every part of it which had come into contact with the culture, this was carefully poured into the distilling flask, the Erlenmeyer flask being finally rinsed into the distilling flask with the minimum of distilled water.

The distilling flask with its contents was then gently boiled in a fume-cupboard. When the frothing had ceased, 10 grm. of potassium sulphate was added to raise the temperature. Boiling was continued until the liquid was practically colourless.

When the flask was cold it was removed from the fume cupboard and about 100 c.c. distilled water was added, together with a few drops of

TABLE I.

Riznlararia Grown in Knop's Solution on Sand.

Number of Flask.	Original Weight of Alga, in gm.	Original Nitrogen-content of Alga, in mg.	Nitrogen-content of Medium and Alga, in mg.	Nitrogen-content of Culture at end of Experiment, in mg.	Actual Gain in Nitrogen, in mg.	Gain in Nitrogen per 100 gm. Alga, in gm.
1. (Control for medium)	—	—	2.8	} Average N-content, 2.71 mg.		
2. "	—	—	2.66			
3. "	—	—	2.66			
4. (Control for alga)	2.712	1.82	} Average—70.89 mg. per 100 gm. alga.			
5. "	2.062	1.54				
6. "	0.159	0.11		4.62	1.80	1.13
7. "	0.120	0.09	2.80	4.76	1.96	1.64
8. "	0.137	0.10	2.81	3.36	0.55	0.40
9. "	0.132	0.10	2.81	3.22	0.41	0.32
10. "	0.096	0.07	2.78	3.08	0.30	0.31
11. "	0.099	0.07	2.78	2.94	0.16	0.16
12. "	0.151	0.11	2.82	4.90	2.08	1.38
13. "	0.104	0.07	2.78	2.52	—0.26	—
14. "	0.087	0.06	2.77	3.78	1.01	1.16
15. "	0.192	0.14	2.85	3.22	0.37	0.19
16. "	0.124	0.09	2.80	3.22	0.42	0.34
17. "	0.225	0.16	2.87	—	—	—

Average increase in nitrogen-content is 639.8 mg. per 100 gm. alga.

TABLE II.

Nostoc Grown in Detmer-Moore Solution on Sand.

Number of Flask.	Original Weight of Alga, in gm.	Original Nitrogen-content of Alga, in mg.	Nitrogen-content of Medium and Alga, in mg.	Nitrogen-content of Culture at end of Experiment, in mg.	Actual Gain in Nitrogen, in mg.	Gain in Nitrogen per 100 gm. Alga, in gm.
1. (Control for medium)	—	—	2.8	} Average N-content, 2.99 mg.		
2. "	—	—	3.08			
3. "	—	—	3.08			
4. (Control for alga)	8.108	4.80	} Average—49.7 mg. N per 100 gm. alga.			
5. "	9.401	3.78				
6. "	1.056	0.53		4.34	0.82	0.08
7. "	1.022	0.51	3.50	5.74	2.24	0.22
8. "	0.814	0.41	3.41	6.02	2.61	0.32
9. "	0.984	0.49	3.48	3.78	0.30	0.03
10. "	0.974	0.48	3.47	4.48	1.01	0.10
11. "	0.787	0.39	3.38	4.34	0.96	0.12
12. "	1.207	0.60	3.59	4.48	0.89	0.07
13. "	1.235	0.61	3.60	6.58	2.98	0.24
14. "	1.091	0.54	3.53	4.34	0.81	0.07
15. "	0.915	0.46	3.45	5.46	2.01	0.22
16. "	1.392	0.69	3.68	—	—	—
17. "	1.475	0.73	3.72	4.48	0.76	0.05

Average increase in nitrogen-content is 139.5 mg. per 100 gm. alga.

TABLE III.

Nostoc Grown in Knop's Solution on Sand.

Number of Flask.	Original Weight of Alga, in gram.	Original Nitrogen-content of Alga, in mg.	Nitrogen-content of Medium and Alga, in mg.	Nitrogen-content of Culture at end of Experiment, in mg.	Actual Gain in Nitrogen, in mg.	Gain in Nitrogen per 100 gm. Alga, in gram.
1. (Control for medium)	—	—	2.52	Average N-content, 2.66 mg.		
2. "	—	—	2.94			
3. "	—	—	2.52			
4. (Control for alga)	8.108	4.80	Average—49.7 mg. N per 100 gm. alga.			
5. "	9.401	3.78				
6. "	1.173	0.58				
7. "	0.904	0.45	3.11	5.60	2.49	0.28
8. "	1.030	0.51	3.17	5.32	2.15	0.21
9. "	0.738	0.37	3.03	3.36	0.33	0.05
10. "	1.494	0.74	3.40	5.04	1.64	0.11
11. "	1.488	0.74	3.40	5.32	1.92	0.13
12. "	1.104	0.55	3.21	3.78	0.57	0.05
13. "	1.808	0.90	3.56	5.60	2.04	0.11
14. "	1.186	0.59	3.25	5.88	2.63	0.22
15. "	1.096	0.55	3.21	5.46	2.25	0.21
16. "	1.115	0.55	3.21	3.92	0.71	0.06
17. "	1.495	0.74	3.40	5.04	1.64	0.11

Average increase in nitrogen-content is 138.7 mg. per 100 gm. alga.

TABLE IV.

Gloeocapsa Grown in Artificial Sea-water on Sand.

Number of Flask.	Original Weight of Alga, in gram.	Original Nitrogen-content of Alga, in mg.	Nitrogen-content of Medium and Alga, in mg.	Nitrogen-content of Culture at end of Experiment, in mg.	Actual Gain in Nitrogen, in mg.	Gain in Nitrogen per 100 gram. Alga, in gram.
1. (Control for medium)	—	—	0.14	} Average N-content 0.21 mg.		
2. "	—	—	0.28			
3. (Control for alga only)	2.113	3.78 =	178.89 mg. per 100 gram. alga.			
4.	0.067	0.12	0.33	2.94	2.61	3.91
5.	0.067	0.12	0.33	2.58	2.25	3.36
6.	0.152	0.27	0.48	2.94	2.46	1.61
7.	0.110	0.20	0.41	2.52	2.11	1.92
8.	0.081	0.15	0.36	1.54	1.18	1.46
9.	0.102	0.18	0.39	2.80	2.41	2.36
10.	0.104	0.19	0.40	2.66	2.26	2.17
11.	0.061	0.11	0.32	2.80	2.48	4.07
12.	0.063	0.11	0.32	2.94	2.62	4.16
13.	0.087	0.16	0.37	2.80	2.43	2.79
14.	0.194	0.35	0.56	3.08	2.52	1.30
15.	0.172	0.31	0.52	3.08	2.56	1.49

Average increase in nitrogen-content is 2550.0 mg. per 100 gm. alga.

methyl orange as an indicator. A saturated solution of sodium hydrate was added until the contents of the flask were just alkaline. When this state was reached, excess of sodium hydrate to the extent of 50 c.c. was added. The contents were then distilled into an Erlenmeyer flask containing 25 c.c. of decinormal sulphuric acid and one drop of methyl orange. Boiling proceeded for 30 minutes, after which the acid in the receiving-flask was titrated against decinormal sodium hydrate solution to estimate the amount of acid neutralized by ammonia which had been liberated. The nitrogen-content of each distilling flask was thus easily calculated.

This procedure was adopted for every flask, and the materials used were identical in quality and quantity.

The results obtained are shown in Tables I, II, III, IV.

From these tables it can be seen that in every case, except No. 13 in Table I, there has been an increase in the nitrogen-content.

A fact to be noted is that *Gloeocapsa*, when grown in artificial seawater, a medium devoid of nitrate (see p. 724) or any combined nitrogen, was able to assimilate a high proportion of gaseous nitrogen, the average figure being 2550.0 mg. per 100 grm. of alga. This figure is far in advance of the other three series, although they, too, gave positive results in the matter of nitrogen fixation. The inconsistency of many of the figures, as, for example, Nos. 6 and 8 in Table II, can possibly be accounted for by the fact that time was not available for the growth of pure strains of the algae, so that there was probably considerable variation in the mass of material used.

MICROSCOPIC EXAMINATION OF ALGAL MATERIAL FOR BACTERIAL ASSOCIATION.

The issue that presented itself as a result of the figures obtained with cultures grown on sand was whether the undoubted nitrogen fixation was brought about by the algae themselves, or by nitrogen-fixing bacteria which might be present in the mucous sheath, which in the Cyanophyceae is typically pronounced. To this end a microscopic examination was made of material prepared by fixing small masses of *Nostoc*, *Rivularia* and *Gloeocapsa* in Bouin's and Flemming's fixatives and in formalin, and staining with a variety of stains, of which Carbol-fuchsin proved to be the most effective. As a result of this investigation a rich bacterial flora was found to occur in the copious mucilage characteristic of the algal types under investigation. The bacterial forms observed included various cocci and bacilli, the varieties associated with all three algae showing similarity among themselves, while some of them showed marked resemblance to well-known nitrogen-fixing types.

CULTURAL EXAMINATION OF ALGAL MATERIAL FOR NITROGEN-FIXING BACTERIA.

The presence of bacteria in the mucous sheaths of the algae having been established, the question arose whether these bacteria might be able to fix atmospheric nitrogen.

In order to ascertain this, it was necessary to grow the algae under conditions favourable to the growth of bacteria—that is, in conditions approximating to those of the soil in temperature and darkness, and in a suitable nutritive medium.

Accordingly, a series of twenty-three Erlenmeyer flasks of 200 c.c. capacity was arranged, each containing 100 c.c. of the following nutritive medium which is suitable for the growth of nitrogen-fixing bacteria :

Glucose	1 gm.
Dipotassium phosphate		0.2 gm.
Magnesium sulphate	0.02 gm.
Calcium carbonate	0.2 gm.
Tap water	100 c.c.

Difficulty was experienced in accurately removing with a pipette the liquid containing the insoluble calcium carbonate, and so the required amount of calcium carbonate, 0.2 gm., was weighed out separately into each flask. The flasks containing the calcium carbonate were plugged and sterilized in an autoclave, fresh plugs of cotton wool being inserted when the flasks were removed from the autoclave.

The solution was made up in concentrated form, i. e. four times normal strength, and sterilized. When it was removed from the autoclave the liquid was seen to have become a golden brown colour. The change was probably the result of the formation of compounds of higher molecular weight by the action of the heat on the glucose.

Flasks of tap water were also sterilized. This was used in preference to distilled water since it might contain traces of iron salts, which would be conducive to bacterial growth.

25 c.c. of the solution and 75 c.c. of water were added to each sterilized flask. The flasks, with the plugs removed, were heated to boiling point, when they were plugged and left to cool.

Twenty of the flasks were inoculated with carefully weighed amounts of *Gloeocapsa*, three flasks being left as controls for the estimation of the nitrogen-content of the medium only. To each of the controls was added 25 c.c. concentrated sulphuric acid. These flasks were put aside for a subsequent estimation of the initial nitrogen-content of the medium.

The flasks, inoculated in the same way as described on page 727, were placed in an incubator at a temperature of 23° C.

Ten days later a scum had begun to appear on the surface of the liquid in the flasks. As was to be expected, in the absence of light the alga ceased to grow and had become a yellowish mass. A further examination nine days later showed that the scum formation had continued.

After sixty-six days the series of flasks was removed from the incubator for the purpose of estimating their nitrogen-content. The liquid in most of the flasks had become gelatinous and turbid. In some cases there was fungal growth.

The contents of the flasks were transferred to distilling flasks and analysed for their nitrogen-content according to the method described on page 727, except that the zinc and salicylic acid were omitted, since no nitrate occurred in the medium. The results obtained are given in Table V.

TABLE V.

Number of Flask.	Original Weight of Alga, in gm.	Original Nitrogen-content of Alga, in mg.	Nitrogen-content of Medium and Alga, in mg.	Nitrogen-content of Culture at end of Experiment, in mg.	Actual Gain in Nitrogen, in mg.	Gain in Nitrogen per 100 gm. Alga, in mg.
1. (Control for medium)	—	—	0.28	Average N-content 0.37 mg.		
2. "	—	—	0.42			
3. "	—	—	0.42			
4. (Control for alga)	3.133	5.88	187.74	Average N-content per 100 gm. alga.		183.13 mg.
5. "	4.001	7.14	178.51			
6.	0.071	0.13	0.50	0.98	0.48	671.8
7.	0.075	0.14	0.51	1.82	1.31	1746.0
8.	0.058	0.11	0.48	1.40	0.92	1587.0
9.	0.074	0.14	0.51	2.10	1.59	2150.0
10.	0.064	0.12	0.49	2.80	2.31	3609.0
11.	0.062	0.11	0.48	1.12	0.64	1209.0
12.	0.105	0.19	0.56	1.98	1.42	1347.0
13.	0.075	0.14	0.51	0.98	0.47	627.0
14.	0.143	0.26	0.63	1.68	1.05	732.0
15.	0.059	0.11	0.48	1.82	1.34	2269.0
16.	0.080	0.15	0.52	1.14	0.62	774.0
17.	0.103	0.19	0.56	1.14	0.58	561.0
18.	0.088	0.16	0.53	1.40	0.87	984.0
19.	0.101	0.19	0.56	1.26	0.70	695.0
20.	0.069	0.13	0.50	1.26	0.76	1102.0
21.	0.147	0.27	0.64	1.68	1.04	706.0
22.	0.076	0.14	0.51	2.10	1.59	2089.0
23.	0.098	0.18	0.55	1.14	0.59	600.0
24.	0.121	0.22	0.59	1.68	1.09	896.0
25.	0.127	0.23	0.60	1.98	1.38	1081.0

Average increase in nitrogen-content is 1268.1 mg. per 100 gm. *Gloeocapsa*.

A similar series of twenty-three flasks was inoculated with *Nostoc* in repetition of the *Gloeocapsa* experiment. At the end of 101 days the series was removed from the incubator in order to estimate the nitrogen-

content of the flasks. That bacterial growth had taken place was evident by the turbidity of the liquid in the flasks and by the scum on the sides. Fungal growth had also occurred in some cases.

The results obtained with this series are shown in Table VI.

TABLE VI.

Number of Flask.	Original Weight of Alga, in gram.	Original Nitrogen- content of Alga, in mg.	Nitrogen- content of Medium and Alga, in mg.	Nitrogen- content of Culture at end of Experiment, in mg.	Actual Gain in Nitrogen, in mg.	Gain in Nitrogen per 100 gram. Alga, in mg.
1. (Control for medium)	—	—	0.28	Average N-content 0.33 mg.		
2. „	—	—	0.28			
3. „	—	—	0.42			
4. (Control for alga)	2.091	7.84	= 375.04 mg. N per 100 gram. alga.			
5.	0.014	0.05	0.38	2.66	2.28	16288.0
6.	0.021	0.08	0.41	2.10	1.69	8067.0
7.	0.041	0.15	0.48	2.94	2.46	5988.0
8.	0.025	0.09	0.42	2.38	1.96	7836.0
9.	0.060	0.23	0.56	2.52	1.96	3280.0
10.	0.027	0.10	0.43	1.54	1.11	4118.0
11.	0.034	0.13	0.46	2.52	2.06	6073.0
12.	0.029	0.11	0.44	0.002	—	—
13.	0.024	0.09	0.42	2.38	1.96	8180.0
14.	0.036	0.14	0.47	2.52	2.05	5715.0
15.	0.033	0.12	0.45	1.54	1.09	3296.0
16.	0.055	0.21	0.54	2.80	2.26	4121.0
17.	0.039	0.15	0.48	1.82	1.34	3453.0
18.	0.045	0.17	0.50	1.40	0.90	2008.0
19.	0.021	0.08	0.41	1.26	0.85	4067.0
20.	0.023	0.09	0.42	1.96	1.54	6726.0
21.	0.037	0.14	0.47	1.96	1.49	4037.0
22.	0.034	0.13	0.46	1.82	1.36	4014.0
23.	0.047	0.18	0.51	2.38	1.87	3993.0
24.	0.030	0.11	0.44	2.94	2.50	8333.0

Average increase in nitrogen-content is 5832.0 mg. per 100 grm. *Nostoc*.

Yet another similar series was started, this time for the investigation of *Rivularia*. The conditions were precisely the same as in the other two series. After 109 days' incubation the flasks were tested for their nitrogen-content. The liquid in the flasks had become turbid, jelly-like, and infected by fungi in some cases.

The figures obtained from the *Rivularia* series are shown in Table VII.

From the figures in Tables V, VI, VII it is seen that with one exception, namely, No. 12 in Table VI, every flask gives an appreciable increase in nitrogen-content. The relatively low nitrogen fixation in these cultures caused some surprise, and the expectation arose that the bacterial growth in the flasks was not as prolific as it might have been, owing, possibly, to the alteration of the glucose under the heat of the autoclave, the

modification of the carbohydrate being possibly less suitable for the growth of bacteria than the original glucose would be.

TABLE VII.

Number of Flask.	Original Weight of Alga, in gm.	Original Nitrogen-content of Alga, in mg.	Nitrogen-content of Medium and Alga, in mg.	Nitrogen-content of Culture at end of Experiment, in mg.	Actual Gain in Nitrogen, in mg.	Gain in Nitrogen, per 100 gm. Alga, in mg.
1. (Control for medium)	—	—	0.56	Average N-content 0.47 mg.		
2. "	—	—	0.42			
3. "	—	—	0.42			
4. (Control for alga)	4.833	7.84	162.28	Average wt. N per 100 gm. alga is 161.88 mg.		
5. "	3.901	6.03	161.55			
6. "	0.079	0.13	0.60		0.52	665.0
7. "	0.122	0.20	0.67	1.40	0.73	603.0
8. "	0.128	0.21	0.68	1.54	0.86	677.0
9. "	0.110	0.18	0.65	3.34	2.69	2450.0
10. "	0.109	0.18	0.65	1.26	0.61	515.0
11. "	0.095	0.15	0.62	2.52	1.9	1999.0
12. "	0.122	0.20	0.67	—	—	—
13. "	0.113	0.18	0.65	—	—	—
14. "	0.110	0.18	0.65	0.84	0.19	177.0
15. "	0.106	0.17	0.64	1.12	0.48	454.0
16. "	0.120	0.19	0.66	1.96	0.13	1083.0
17. "	0.103	0.17	0.64	1.40	0.76	745.0
18. "	0.077	0.13	0.60	1.82	1.22	1595.0
19. "	0.127	0.21	0.68	1.68	1.00	793.0
20. "	0.098	0.16	0.63	1.68	1.05	1076.0
21. "	0.117	0.19	0.66	1.54	0.88	756.0
22. "	0.143	0.23	0.70	1.54	0.84	588.0
23. "	0.119	0.19	0.66	1.54	0.88	740.0
24. "	0.121	0.20	0.67	1.40	0.73	609.0
25. "	0.162	0.26	0.73	2.42	1.69	1044.0

Average increase in nitrogen-content per 100 gm. *Rivularia* is 902.5 mg.

In order to test this, two further series of incubations were started precisely similar to those previously described, with the exception that in these cases the glucose medium was only boiled to ensure sterility, and not autoclaved. This procedure prevented any alteration of the glucose, and the liquid remained colourless.

Nostoc and *Gloeocapsa* were used for the purpose of inoculating the two series, and the results obtained after 104 and 84 days' incubation respectively are shown in Tables VIII and IX.

A comparison of the figures in these tables with those in Tables V and VI would suggest that the autoclaved medium, with its subsequent coloration due to the modification of the glucose, is more favourable to this particular bacterial growth than is the solution when it is boiled only. But it must be borne in mind that the algal materials used for both series

were not identical, and so the results are not absolutely conclusive. The material used for Series VIII and IX was obtained much later than that used in Series V and VI, and it was less firm in consistency and not so healthy in appearance. However, these results summarized in Tables VIII and IX add further testimony to the fact that there is an appreciable increase in the nitrogen-content when these algae are incubated under conditions favourable for bacterial growth.

TABLE VIII.

Number of Flask.	Original Weight of Alga, in grm.	Original Nitrogen-content of Alga, in mg.	Nitrogen-content of Medium and Alga, in mg.	Nitrogen-content of Culture at end of Experiment, in mg.	Actual Gain in Nitrogen, in mg.	Gain in Nitrogen per 100 grm. Alga, in mg.
1. (Control for medium)	—	—	0.43	Average N-content 0.52 mg.		
2. "	—	—	0.57			
3. "	—	—	0.57			
4. (Control for alga)	6.307	5.22	Average wt. N per 100 grm. alga = 73.85 mg.			
5. "	7.140	4.63				
6. "	0.422	0.31				
7.	0.116	0.09	0.83	3.87	3.04	718.0
8.	0.347	0.26	0.78	2.69	1.91	550.4
9.	0.246	0.18	0.70	2.55	1.85	747.9
10.	0.401	0.30	0.82	2.97	2.15	533.7
11.	0.444	0.33	0.85	2.41	1.56	346.8
12.	0.142	0.11	0.63	1.85	1.22	859.2
13.	0.183	0.14	0.66	1.99	1.33	726.7
14.	0.342	0.25	0.77	1.71	0.94	271.9
15.	0.161	0.12	0.64	2.41	1.77	1093.1
16.	0.198	0.15	0.67	1.71	1.04	520.2
17.	0.177	0.13	0.65	2.69	2.04	1146.8
18.	0.366	0.27	0.79	3.53	2.74	745.9
19.	0.298	0.22	0.74	2.83	2.09	697.9
20.	0.339	0.25	0.77	3.39	2.62	769.9
21.	0.249	0.18	0.70	1.85	1.15	456.2
22.	0.252	0.19	0.71	1.43	0.72	285.7
23.	0.282	0.21	0.73	4.23	3.50	1237.5
24.	0.292	0.22	0.74	0.73	—	—
25.	0.278	0.21	0.73	1.71	0.98	350.7

Average increase in nitrogen-content per 100 grm. *Nostoc* is 723.1 mg.

In order to test the accuracy of the method adopted for estimating the nitrogen-content, 0.1 grm. of pure urea was dissolved in 100 c.c. of distilled water, and 10 c.c. of the solution was transferred to each of three distilling flasks. The nitrogen-content of these was determined in the manner already described, precisely similar quantities of reagents being used throughout. The results are shown in Table X.

The uniformity of these results, together with the fact that the error was always on the same side, points to the conclusion that the error is due

to the presence of nitrogenous substances in the reagents used. Since all the estimations, including the controls, were carried out with materials identical in quality and quantity, it is obvious that precisely the same error will be introduced into each estimation made, so that the final results will be absolutely comparable.

TABLE IX.

Number of Flask.	Original Weight of Alga, in grm.	Original Nitrogen-content of Alga, in mg.	Nitrogen-content of Medium and Alga, in mg.	Nitrogen-content of Culture at end of Experiment, in mg.	Actual Gain in Nitrogen, in mg.	Gain in Nitrogen per 100 grm. Alga, in mg.
1. (Control for medium)	—	—	0.29	} Average N-content is 0.36 mg.		
2. "	—	—	0.43			
3. (Control for alga)	6.300	6.10	} Average wt. N per 100 mg. alga = 98.4 mg.			
4. "	14.688	14.69				
5.	0.250	0.25	0.61	1.40	0.79	318.0
6.	0.439	0.43	0.79	1.78	0.99	225.0
7.	0.363	0.36	0.72	1.67	0.95	263.6
8.	0.362	0.36	0.72	2.49	1.77	490.9
9.	0.693	0.68	1.04	3.18	2.14	309.2
10.	0.598	0.59	0.95	2.77	1.82	305.0
11.	0.486	0.47	0.83	3.60	2.77	571.2
12.	0.936	0.92	1.28	3.72	2.44	260.6
13.	0.285	0.29	0.65	3.18	2.53	889.8
14.	0.430	0.42	0.78	2.63	1.85	430.0
15.	0.289	0.28	0.64	3.60	2.96	1022.8
16.	0.355	0.35	0.71	2.35	1.64	461.1
17.	0.280	0.27	0.63	2.23	1.60	570.0
18.	0.335	0.33	0.69	3.04	2.35	700.6
19.	0.330	0.32	0.69	2.23	1.54	468.8
20.	0.581	0.57	0.93	2.35	1.42	244.8
21.	0.504	0.49	0.85	3.60	0.75	544.8
22.	0.501	0.49	0.85	3.18	2.33	464.9
23.	0.603	0.59	0.95	3.46	2.51	416.1
24.	0.426	0.42	0.78	3.18	2.40	563.1

Average gain in nitrogen-content per 100 grm. *Gloeocapsa* = 458.6 mg.

TABLE X.

No. of Flask.	N-content in mg.	Known N-content in mg.	Actual Error.	% Error.
1.	4.91	4.67	+0.24	+4.88
2.	4.91	4.67	+0.24	+4.88
3.	4.91	4.67	+0.24	+4.88

Since the error is introduced twice over in estimating the nitrogen-content of the controls in most series, once in determining the nitrogen of the medium, and again in estimating that of the inoculum, it follows that the initial nitrogen allowed for in the cultures is too high by twice the

amount of the error. The final nitrogen will be too high by once the amount of the error; and, therefore, the gain in nitrogen reported, i.e. the difference between the initial and the final nitrogen, will be lower than the actual gain by once the amount of the error. The figures calculated are therefore too low, rather than too high, by the operation of the error, and the constancy of the figures given for the three flasks of the series under discussion at the moment is proof of the reliability of the method.

The conditions of darkness prevailing in the series V, VI, VII, VIII, and IX, which were incubated, were so unfavourable for the growth of the algae that they died off, and so the nitrogen fixation could not have been carried out by them. The evidence obtained points to a fixation of nitrogen by bacterial organisms present in the algal mucous sheath. In order to prove conclusively that this nitrogen fixation was the work of bacteria, it was necessary to isolate the bacteria associated with the algae and to test their powers of nitrogen-fixation.

ISOLATION AND IDENTIFICATION OF BACTERIA ASSOCIATED WITH THE ALGAE.

The method employed for the identification of the bacteria was that of plate cultures. The nutritive medium employed was the same as that used for the series incubated (p. 731), with the addition of 2 per cent. of agar-agar. Petri dishes containing this medium were sterilized in an autoclave at 140° C. When cool and solid, these were inoculated, in a sterile chamber, each with three small masses of alga. Four plates of each alga were completed in this way, and the twelve were placed in an incubator at approximately summer soil-temperature, i.e. about 25° C. The dishes were incubated upside down in order that any moisture given off by the alga would not lie on the agar and thus cause a spreading over the surface of the plate of any bacterial growth obtained. Seven days later around each algal inoculum there were bacterial colonies of considerable size. Many of the plates were contaminated by fungal growth, obviously due to spores or hyphae present in the algal mass.

The bacterial colonies appeared whitish and transparent, and the calcium carbonate in the agar around them had been dissolved, presumably by the action of acids secreted by the bacteria. Some colonies from all the plates were examined microscopically, and a predominance of bacilli of various forms, including long 'beaded' rods and also various forms of cocci, were noted. Subcultures were made from these plates in an attempt to obtain pure colonies, care being taken to avoid subculturing from any colonies which were contaminated by fungal growth. This procedure, of initial culturing followed by successive subculturings of the bacteria, was repeated continually both on this glucose-agar medium and on a similar agar medium containing mannite in substitution for the glucose, with the result

that the following bacterial forms were found to occur consistently: (a) *Azotobacter chroococcum*, (b) a small diplococcus, (c) a very small coccus, (d) *Clostridium pastorianum* (*Bacillus amylobacter*—A. M. et Bred.), (e) forms of *Bacillus mesentericus* (some of which are known to be capable of nitrogen fixation), (f) *Bacillus radicola*, and (g) various forms of bacillus. Those of most frequent occurrence were *A. chroococcum*, *B. radicola*, and *C. pastorianum*. The nitrogen-fixing power of these three predominant forms is established beyond doubt, and, in the limited time available, it was impossible to make an examination of the nitrogen-fixation of any of these forms except those included under (g). Five of these forms, which were labelled B 1 to B 5, were investigated by incubating them in the glucose medium for periods varying from 29 to 121 days, after which the contents of these flasks, together with those of the control flasks, were analysed for their nitrogen-content by the modified Kjeldahl process used and described previously. The results obtained are summarized in Table XI as follows:

TABLE XI.

Number of Flask.	Contents of Flask.	Nitrogen-content at start of Experiment, in mg.	Nitrogen-content at end of Experiment, in mg.	Gain in Nitrogen, in mg.	Average gain in Nitrogen, in mg.
1.	Medium and B 1 killed by sulphuric acid (control)	0.45	0.45		
2.	Medium and B 1 culture	0.45	0.60	0.15	0.15
3.	" "	0.45	0.60	0.15	
4.	" "	0.45	0.60	0.15	
5.	Medium and B 2 killed by sulphuric acid (control)	0.45	0.45		
6.	Medium and B 2 culture	0.45	0.74	0.29	0.71
7.	" "	0.45	0.60	0.15	
8.	" "	0.45	2.14	1.69	
9.	Medium and B 3 killed by sulphuric acid (control)	0.43	0.43		
10.	Medium and B 3 culture	0.43	0.56	0.13	0.07
11.	" "	0.43	0.29	—	
12.	" "	0.43	0.29	—	
13.	Medium and B 4 killed by sulphuric acid (control)	0.43	0.43		
14.	Medium and B 4 culture	0.43	0.56	0.13	0.07
15.	" "	0.43	0.43	0	
16.	" "	0.43	0.71	0.28	
17.	" "	0.43	0.56	0.13	0.13
18.	" "	0.43	0.43	0	
19.	Medium and B 5 killed by sulphuric acid (control)	0.43	0.43		
20.	Medium and B 5 culture	0.43	0.71	0.28	0.13
21.	" "	0.43	0.56	0.13	
22.	" "	0.43	0.56	0.13	
23.	" "	0.43	0.43	0	0.13
24.	" "	0.43	0.56	0.13	
25.	" "	0.43	0.56	0.13	

As the results in Table XI show, in no case was the fixation of nitrogen by these bacteria appreciable. In each case, except that of B 3, a slight fixation was apparent, but the amount of it was not sufficiently great to warrant the assumption that these organisms are definite nitrogen-fixers without further experimental evidence. It would seem probable that these organisms may act in conjunction with the known nitrogen-fixers present—that is to say, they may act as ‘helpers’ in the fixation of atmospheric nitrogen, their exact rôle being unknown. Lipmann (7) suggested that such bacteria occurring in his cultures—bacteria which he named as B 36, B 37, B 30, B 34, B 35—may reduce the acidity of the medium to that required by the nitrogen-fixing organism. He states that although they are capable of fixing only very minute quantities of nitrogen, if any, themselves, when in co-operation with species of *Azotobacter* they are able to enhance the nitrogen-fixing power of these latter organisms.

CONCLUSION.

The outstanding fact which has been revealed by the foregoing investigations appears to be that nitrogen-fixing bacteria are present in the copious mucilage associated with members of the Cyanophyceae. That they are present in such numbers as to effect appreciable fixation of atmospheric nitrogen is supported by the figures of nitrogen-fixation summarized in Tables V–IX.

When these facts are considered it seems feasible to postulate an association of nitrogen-fixing bacteria (and, possibly, other bacteria which aid these in their metabolism) and some Cyanophyceae, namely, species of *Nostoc*, *Rivularia*, and *Gloeocapsa*. The bacteria inhabit the mucous sheath of the alga, and, in this way, are assured of protection, moisture, and probably carbohydrate. Here they build up compounds of nitrogen assimilated from the air, and secrete any surplus into the algal sheath. In this way the algae are supplied with nitrogenous material, and are able, consequently, to flourish in regions lacking in some of the ordinary plant food-materials.

In conclusion, I wish to express indebtedness to Dr. F. A. Mockridge for suggesting this work, and for much advice and help in carrying it out. Acknowledgements are also due to the authorities of the University College of Swansea for the award of the Singleton Exhibition, during the tenure of which the work was completed.

SUMMARY.

The prolific growth of Cyanophyceae in regions apparently lacking the ordinary plant food-materials prompted the investigation. Three members of the group were selected for investigation, namely, species of *Nostoc*, *Rivularia*, and *Gloeocapsa*.

1. A preliminary investigation was made to ascertain the artificial

media best suited to the growth of these algae. The efficiency of four solutions—Detmer-Moore, Knop, artificial sea-water, and natural sea-water—were tested in three ways: (*a*) with sand forming sand-slopes, (*b*) with agar-agar forming a solid medium, and (*c*) alone, giving a liquid medium.

2. From results obtained in 1, the following algal cultures were started:

Rivularia in Detmer-Moore solution on sand.

Nostoc in Detmer-Moore solution on sand.

Nostoc in Knop solution on sand.

Gloeocapsa in artificial sea-water on sand.

After some time the contents of the flasks were tested for nitrogen, and an appreciable increase was found in the four series.

3. Microscopic examination of the fixed and stained algae revealed the presence of bacteria in the algal mucus.

4. Series were incubated to ascertain whether the bacteria noted in 3 were able to fix atmospheric nitrogen, with the result that considerable fixation of atmospheric nitrogen by the bacteria collectively was established.

5. An attempt was made to isolate and identify the forms constituting this mixed bacterial flora. Some well-known nitrogen-fixing organisms, such as *A. chroococcum*, *C. pastorianum*, and *B. radicola*, were present in large numbers.

Other bacteria were tested directly for their powers of nitrogen fixation, and in some cases slight fixation was recorded.

The conclusion was drawn that nitrogen-fixing bacteria inhabit the mucus of some Cyanophyceae to the mutual benefit of both organisms concerned.

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The Biology of Banana Wilt (Panama Disease).

I. Root Inoculation Experiments.

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With thirty-five Figures in the Text.

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INTRODUCTION.

IT is not always that the first outbreak of a disease can be traced with certainty. This is true of Banana Wilt disease or, as it is generally called, Panama Disease. While it may have made its appearance in the Western Hemisphere for the first time towards the close of last century, its wide distribution in the Old World suggests a much greater antiquity. It is worthy of note that the disease became economically significant, or at least was given attention, about the same time that the banana industry had commenced its rapid expansion. According to Brandes (2), the first mention of the disease was made by Higgins (8) in Honolulu in 1904, and McKenney (10) also states that it was already important in Costa Rica and Panama about the same time. Since 1910 the problem has received considerable attention at the hands of pathologists.

This work is admirably summarized in the paper by Brandes (2) (1919), where he quotes from a bibliography of thirty-three titles. Since then Panama Disease has been the subject of a very considerable number of memoirs and departmental reports. In short, it has now been reported from practically all countries where bananas are grown.

The important results to date are the outcome of work by E. F. Smith (13), who named the organism and indicated its capacity for growth in the vascular tissues, Ashby (1), who isolated and carefully described it in pure culture, and Brandes (2), who carried out detailed inoculation experiments whereby its pathogenicity was conclusively demonstrated. This important work has been repeated and verified by Hansford (6) and Reinking (11). The more recent research of the last two authors has been concerned with detailed systematic studies of the genus *Fusarium* (7, 12).

For all practical purposes our knowledge of Panama Disease may be briefly summarized as follows: It is a wilt disease of great severity and is associated with the parasitic soil organism, *Fusarium cubense*, on suckers and roots. Several varieties are highly susceptible, but of outstanding commercial importance is the Gros Michel banana (*Musa sapientum*). While the various inoculation experiments described give a clear proof of the pathogenicity of *F. cubense*, in no case do possible accessory environmental conditions, such as soil moisture, aeration, texture, acidity, humus and mineral supply, appear to have been adequately considered. The several experimental records show great uniformity of method, namely, that sterilized suckers in containers of sterilized soil were inoculated with *F. cubense*, and that proofs of pathogenicity after nine or more months were obtained. Thus, while the beginning and end points of the experiments are usually described in detail, the records contain no information regarding intermediate events. Further, there have been no detailed experiments to test whether the attacking power of the organism is influenced by modification of the various external factors. In our present state of knowledge, then, there are two definite points of view to be considered.

1. The disease may be due entirely to internal factors, i. e. to genetic constitution, which determines lack of resistance to *F. cubense*.
2. The disease may be due to predisposing external factors which adversely affect the health of the plant, so rendering it susceptible.

The importance of genetic constitution in this particular instance has long been known since the Canary banana (*Musa cavendishii*), the Lacatan, and others, will grow on land where the Gros Michel has already succumbed to disease. The point to be determined is whether this well-known susceptibility is constant for all external conditions, or whether it is possible to delay or prevent infection by modifying the bad soil conditions under which the plant is frequently grown. At the present time there is much to be said for both points of view.

On broad ecological grounds there are many reasons why possible external soil factors should receive careful consideration. Such factors include (1) water supply, (2) air supply, (3) temperature, (4) supply of nutrients, (5) various injurious factors. Of these, on *a priori* grounds, the first and second appear to be of greatest importance. It is true that temperature has been shown to be important in other wilt diseases caused by species of *Fusarium*, but in the typical Central American plantation it seems unlikely that this factor is in operation. Field evidence, on the other hand, points to water-supply and aeration as factors which merit close attention. Thus there is very little disease in the Parish of St. Catherine's in Jamaica, and none in Colombia, where all the banana lands are irrigated, i. e. where plants are grown under conditions of controlled soil moisture. On the other hand, disease is of frequent occurrence on stiff soils in regions of high rainfall, and it also occurs on open light sandy soils. Both of these soil types allow of great variation in moisture supply to the roots.¹ The state of soil aeration also deserves careful attention since most of the untiled virgin soils, of compact alluvium or clay, are inadequately ventilated, as indicated by their high degree of mottling. On the fourth factor, supply of nutrients, it is not profitable to comment at the present time. In so far as *F. cubense* is mainly a wound parasite, usually entering through cut surfaces, any wounding agency, such as weevil-borer (*Cosmopolites sordidus*) may constitute an important accessory factor.

It is not necessary here to discuss all the implications of a test case such as this, i. e. external *versus* internal factors, but it is clear that if it proves to be purely a matter of the hardihood of the plant as determined by genetic constitution, then no effort should be spared to produce an immune banana to replace the Gros Michel. On the other hand, should the disease be found to depend on the presence of external soil factors susceptible of modification, then there will be a tendency in time, as determined by economic considerations, to make for progress in the treatment and improvement of tropical soils.

In investigating the parasitic attack of *F. cubense* on the banana, there are two distinct though interrelated pathological problems, namely, root-infection and sucker-infection. The present work is concerned with the former.

I. ROOT INOCULATION EXPERIMENTS.

Panama Disease progresses from the sucker into the aerial parts of the plant, infection originating according to previous investigators either at the cut end of the sucker (where it has been separated from the parent rhizome),

¹ At an early stage in this investigation Professor H. R. Briton-Jones called my attention to the probable importance of variable soil moisture in relation to infection.

or in the roots. With the exception of a brief record by Brandes (2), little attention has been given to this subject. According to him the unwounded young roots and wounded rhizome are the natural infection courts. His experimental method consisted in washing roots free of soil, applying a spore suspension, and covering with moist cotton wool. Roots were examined for penetration after twelve hours. In the case of the thread-like roots there was no evidence of penetration, but positive results were obtained, when the apices of young fleshy roots were similarly exposed and inoculated. He states that while the epidermis and cortex were deeply penetrated, root-hairs were not attacked. He also considers that proof that young uninjured fleshy roots act as infection courts is amply furnished by these experiments. The writer of the present paper, while he admits that fungal penetration could be obtained by the experimental method adopted, has found that root infection does not take place under all conditions—a view that is supported, not only by direct experiment, but also by ample field evidence. The author has frequently had cause to remark on the fact that badly infected plants with well-marked external and internal symptoms had many roots in which superficial blemishing and vascular discoloration (an important wilt symptom) were absent. Further, from a physiological stand-point, it will be admitted that a young root from which soil has been washed, and which has been exposed for a short period, even with the most delicate manipulation, cannot be regarded as either uninjured or free from shock.

To throw light on these questions the following inoculation experiments were undertaken to test the pathogenicity of *F. cubense* to the roots of the Gros Michel.

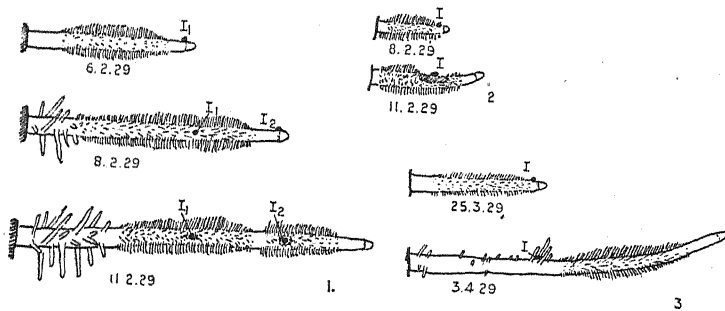
II. INOCULATION EXPERIMENTS IN VENTILATED MOIST CHAMBERS.

A first series of experiments was carried out as follows:

Cylindrical tin boxes of 4,000 c.c. capacity, with perforated lids, were lined with blotting paper and kept moist by the addition of sterilized water. Into these moist chambers banana suckers (sterilized on the outside by immersion in 0.2 per cent. mercuric chloride solution for ten minutes, with subsequent washings in sterilized water) were placed. After some days roots pushed out from various points on the suckers. These grew rapidly under the conditions provided, and as a rule were abundantly covered with root-hairs. Inoculations were made by gently placing a small piece of a culture of *F. cubense* (growing on malt, potato, or rice agar) just at the edge of the root-cap. The use of a solid inoculum made it more easy to follow the subsequent changes.

A large number of such inoculations were carried out over a period of three months with varying results. In the case of stout, strongly growing roots negative results were obtained. A characteristic record is shown in

Fig. 1. A root 4.5 cm. long by 0.4 cm. diameter, with well-developed root-hairs and white in colour, was inoculated at the tip with *F. cubense* growing on rice-agar. After two days the root had elongated by 2.5 cm., so that the inoculum was left at that distance behind the apex. It was again inoculated at the apex, and on examination three days later the second inoculum was 2.4 cm. behind the apex. The root was not discoloured in any way at either point, and microscopical examination did not



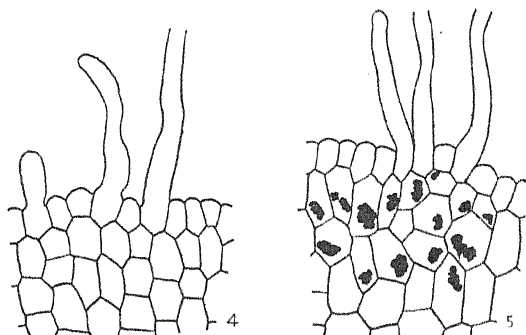
FIGS. 1-3. 1. Inoculation of a root-tip of a healthy Gros Michel root grown in a ventilated moist chamber—no infection has resulted. $\times 2/3$. I. = inoculum. 2. Inoculation under the same conditions of a slender, slow-growing root. A blemish has been produced round the inoculum. $\times 2/3$. 3. Inoculation of Gros Michel root in ventilated moist chamber—no infection. Rootlet formation has been induced at the point of inoculation. $\times 2/3$.

reveal damage to the superficial tissue or entrance of the fungal hyphae. Altogether some thirty such records were made, and in all cases where the roots were stout and growing vigorously negative results were obtained. Variation in the inoculating medium used gave minor differences, such as slight superficial colouring. The cultures used were typical isolations of *F. cubense*.

Under the same experimental conditions some slender, puny, and slow-growing roots were inoculated. After three days (Fig. 2) the root had grown 1.3 cm., leaving the inoculum at that distance from the apex. Round the inoculum, however, there was a dark coloured blemish, accompanied by a local flattening of the root-hairs, but the injury sustained was not sufficient to kill or cause complete cessation of growth. Superficial blemishes of this kind, accompanied by a reddish colouration, were frequently obtained from inoculations, but they were not observed to develop into more than slight local injuries.

In another similar series of root inoculations, *F. cubense*, growing on a synthetic medium rich in asparagin, was used. No infection took place (Fig. 3), but after nine days it was found that diffusion of substances from the inoculum into the tissues of the root had stimulated the formation of lateral rootlets, so that a cluster of them was found growing round and through the inoculum. Other rootlets which appeared elsewhere were

decidedly smaller than those in the vicinity of the inoculum. This observation was constant for the six roots used in this experiment. That diffusion of substances from the inoculum, or from invaded tissue, has marked stimulating effects has been frequently demonstrated in the course of this work. Transverse sections through the roots at the points of inoculation revealed distinct protoplasmic changes. The sub-epidermal cells had not only



FIGS. 4 and 5. 4. Piliferous layer and superficial tissues of normal Gros Michel root in transverse section. $\times 210$. 5. Piliferous layer and superficial tissues of root near inoculum. The diffusion of fungal secretions has led to coagulation of cell contents. $\times 210$.

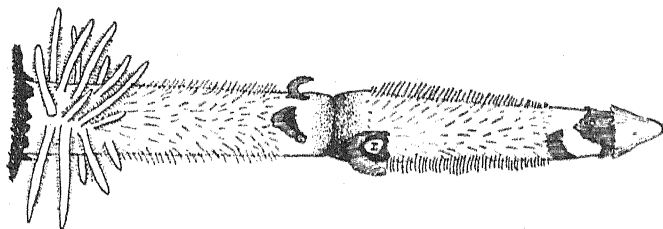


FIG. 6. Inoculated small Gros Michel root after eleven days. The inoculum at t, originally the apex, has caused rootlet formation at the base and discoloration and constriction near the inoculum. Growth, however, has continued. $\times 3$.

acquired an unusually distinct granular protoplasm (in contrast to the normal hyaline protoplasm), but also contained masses of a coagulated tannin-like substance—an effect which extended from two to six cells deep (Figs. 4 and 5). That the influence of the foreign substance was felt as far as the stele was indicated by the presence of a brown coagulated substance in the protoxylem vessels on the side towards the inoculum, as well as by the emergence of rootlets already described. Both cortical and stelar tissues on the side away from the inoculum were normal.

When the same inoculum (i. e. *F. cubense* on an asparagin rich medium) was applied to small, slender, and slow-growing roots a characteristic effect, repeated in several instances, was obtained. The root, about 1.5 cm. long, was inoculated at the tip (Fig. 6). The cessation of growth which

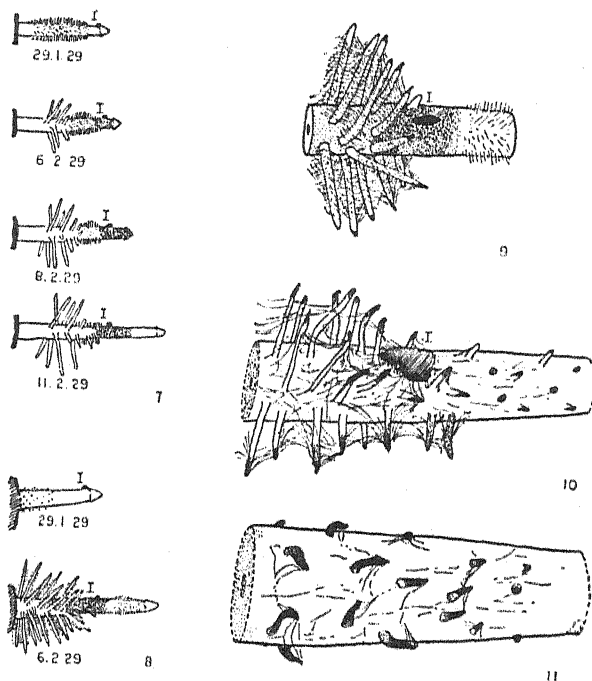
resulted was accompanied by a dark reddish discolouration round the inoculum. This inhibition of apical growth had the same effect as root pruning, and led to the emergence of a large number of basal rootlets. This was followed by a period of slow growth of the main apex, so that by the eleventh day the root was only 2.9 cm. long, with a constricted ring and dark colour round the point of inoculation. As far as could be determined, little decay of tissue or fungal penetration had taken place, the changes recorded having apparently been due to the diffusion of toxic substances from the inoculum.

III. INOCULATION EXPERIMENTS IN UNVENTILATED MOIST CHAMBERS

A series of experiments was also undertaken in which the containers described above were used with unperforated lids. In this way high humidity was combined with an increasing concentration of the carbon dioxide of respiration. Inoculated roots had thus to contend with one important adverse factor, namely, CO_2 concentration. In one experiment sixteen roots were inoculated behind the root cap with *F. cubense* grown on malt agar. After two to four or more days they showed a localized brownish-red blemish in the vicinity of the inoculum. At this stage the most noticeable effect, in contrast to roots inoculated in ventilated containers, was the slowing down or cessation of apical growth, accompanied by the production of a cluster of basal rootlets. As a rule the root-tips became dark in colour, but after a few days the more vigorous roots continued to grow, so that the position of the inoculum was denoted by a discoloured ring, or where the check had been more severe by a local constriction. The less vigorous roots stopped growing, became more markedly affected, and finally succumbed to a gradual dying-back from the apex. Characteristic records are shown in Fig. 7 and Fig. 8. Under these conditions, the root-tips of the fine lateral rootlets proved readily susceptible, and hyphae which soon spread to the tips caused a die-back (Figs. 9 and 10). Rootlets were sometimes attacked as soon as they emerged (Fig. 11). That the effect of *F. cubense* under these conditions is slow and not necessarily completely destructive to the main root is indicated by the growth of rootlets in proximity to the inoculum.

Roots which were dying back under the influence of the fungus were examined microscopically at the end of ten days. Hyphae were observed in the cortex and stele, while the latter was discoloured to a considerable extent. A characteristic purplish colour was commonly found at the junction of living and dead tissue. Depositions of tannins and other substances were found in attacked tissue, while evidence that meristematic cells were being stimulated to growth was also obtained.

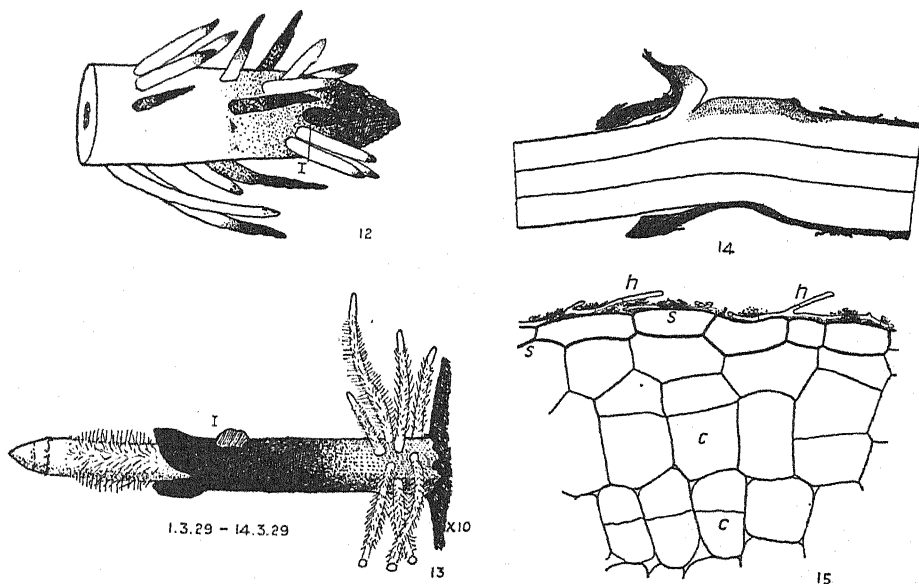
The parasitic activity of *F. cubense* on Gros Michel roots grown in closed chambers was found to be variable. All conditions between complete killing and temporary growth stoppage were found. The following examples may be quoted in illustration. An inoculated root after some days showed the typical cessation of apical growth, darkening of superficial



FIGS. 7-11. Gros Michel roots grown in unventilated moist chambers, inoculated with *F. cubense*. I. = inoculum. 7. The inoculum causes discolouration, cessation of apical growth, and the formation of basal rootlets. The root apex is not killed and growth ultimately continues. $\times 2/3$. 8. A similar record. $\times 2/3$. 9. Infection of delicate lateral root-tips from inoculum on main root. $\times 3$. 10. Spread of *F. cubense* among the rootlets causing apical die-back. $\times 4$. 11. Spread of *F. cubense* along the main root. The latter is not being penetrated, but the delicate tips of lateral rootlets are being killed sometimes at the point of emergence. $\times 7$.

tissues, and production of basal rootlets. In this state it remained for several days, and it appeared as if a gradual dying-back was about to take place. By the fifteenth day after inoculation, however, rootlets began to appear in acropetal succession, and, as indicated in Fig. 12, approached almost to the inoculum itself. The rootlets subsequently became diseased. A second example of such inconclusive inoculation experiments was as follows. A small tip was inoculated with *F. cubense* on potato-agar. As before, the presence of the inoculum led to the cessation of apical growth, to a marked discolouration spreading backwards, and to the formation of basal rootlets. This condition continued for some time, but on the eleventh day apical growth recommenced, the old root-cap was burst through and

partially sloughed off in the process, and by the thirteenth day a short length of new root, covered with root-hairs, had been formed (Fig. 13). Longitudinal sections (Fig. 14) showed that the internal cortical and stelar tissues were healthy, while the outer cortical tissue, which had been invaded by hyphae, had been sloughed off by the development of a cambiform layer



FIGS. 12 and 13. 12. Inconclusive inoculation of Gros Michel root in unventilated moist chamber. While apical growth has ceased, as the result of inoculation at I., complete killing has not taken place, as evidenced by rootlet formation close to the inoculum. $\times 8$. 13. Similar to Fig. 12. The inoculum at I. caused temporary stoppage of apical growth, discolouration, and rootlet formation. Growth continued, however, whereby the old root cap and diseased superficial tissues were burst through and sloughed off. $\times 7$.

FIGS. 14 and 15. 14. Longitudinal section of root shown in Fig. 13. The diseased tissue round the point of inoculation has been sloughed off by the suberization of underlying healthy cells. The stippled area indicates the hypertrophied suberized tissue. $\times 10$. 15. Part of the healthy tissue underlying the sloughed-off diseased tissue. The outer walls are strongly suberized (S.). Note also the cambiform cell divisions (c.), Fungal hyphae (h.) are seen outside but not inside the suberized cambiform layer. $\times 130$.

of tissue whose outer walls were suberized. Fig. 15 shows in detail some of the meristematic cells, which have divided by tangential walls in a cambiform manner, abutting on invaded tissue. These and adjacent cells had walls which were demonstrated by staining and dissolving tests to be suberized. This cambiform layer, which was observable megascopically as a noticeable hypertrophy, was quite free from hyphae in contrast to the dead outer tissue whose cells were full of the hyphae and chlamydospores of *F. cubense*. Similar observations were made on other roots.

In these experiments in closed moist chambers the cessation of root-growth cannot be attributed to the presence of the fungus alone, since uninoculated controls after some time also showed a slowing down or

stoppage of growth, with the development of a pale brown colour. In the absence of a parasitic organism, however, no die-back took place during the time occupied by the experiment.

The evidence from the two contrasted series of experiments described above indicates that CO_2 vitiation is a factor which promotes infection of Gros Michel roots by *F. cubense*. A factor exceedingly difficult to assess, but responsible for the variable and often inconclusive results obtained, is the physiological status of individual roots. It has been clearly demonstrated that actively growing roots do not become infected.

Roots of the Gros Michel, inoculated at some distance behind the apex with *F. cubense* on malt agar, showed a local discolouration, but there was no parasitic effect. This is in keeping with the finding of Brandes.

IV. OBSERVATIONS ON INOCULATED DECAPITATED ROOTS.

Roots of the Gros Michel were grown in closed moist chambers. When they had attained a length of 4 cm. to 5 cm. their tips were cut off

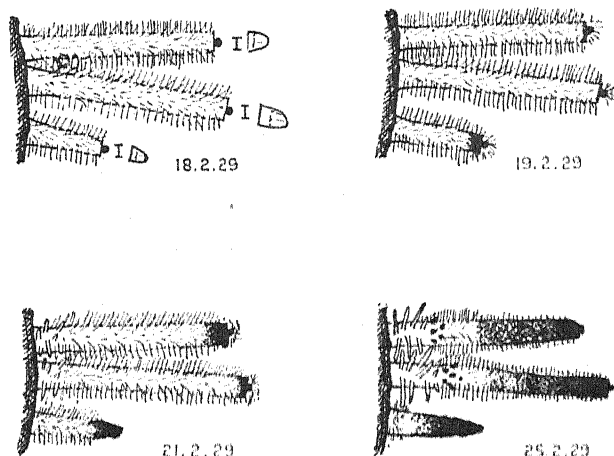
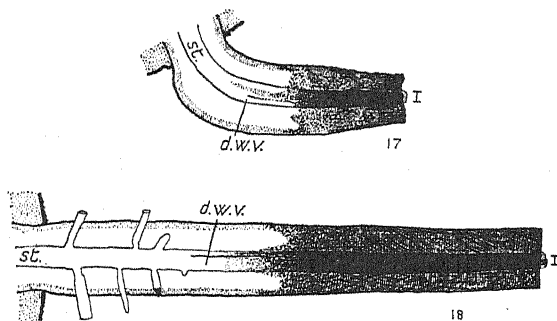


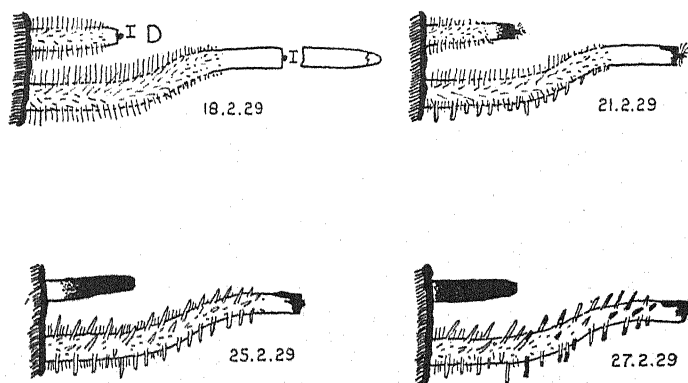
FIG. 16. Inoculation of decapitated Gros Michel roots grown in moist chambers.
 $\times 2/3$. I. = inoculum.

with a sharp scalpel and the cut ends inoculated. Eight roots of varying lengths were treated in this way, and from the detailed observations made every second day it was found that the younger the root the quicker was the decay brought about by *F. cubense*. At the end of the first day the smallest root had a limited dark zone at the inoculated surface partly due to plant juices liberated by cutting and partly to fungal action. After three days the roots showed a decayed area 0.3 to 0.6 cm. in length, and small lateral rootlets began to appear on the larger roots. At the end of seven days the amount of decay varied in different roots from 0.6 to 2.0 cm.,

and rootlets also began to show infections. A characteristic record is shown in Fig. 16. Longitudinal sections showed that hyphae were abundantly present in the terminal tissues and that killing by the diffusion of toxic secretions had taken place in advance of the hyphae. This was more rapid



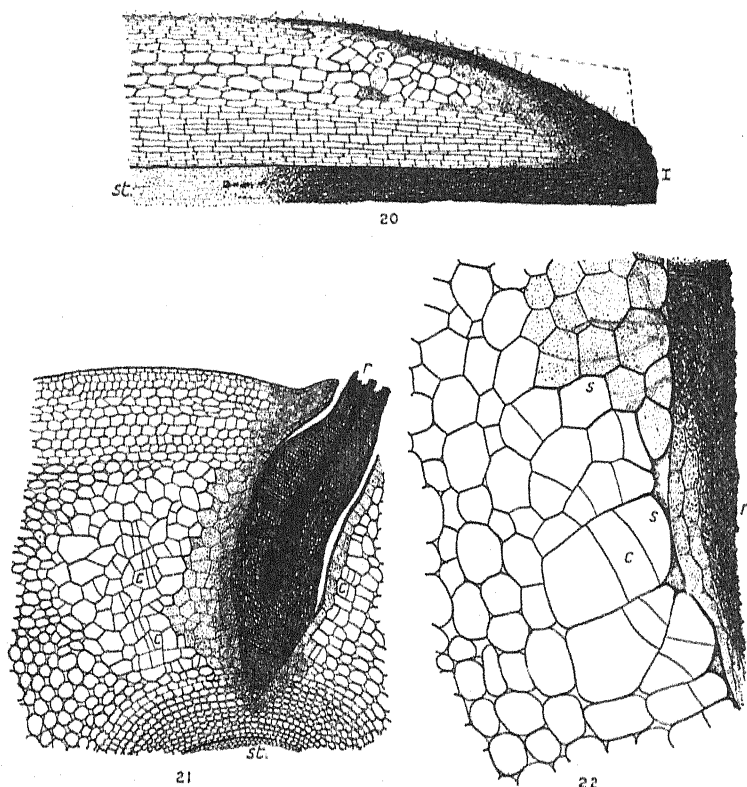
FIGS. 17 and 18. Longitudinal sections of a young and older inoculated decapitated Gros Michel root. The terminal portions of cortex and stele (*St.*) are badly diseased, the progress of the disease being more rapid along the latter. At this stage characteristic yellow and red colours may be seen in wood-vessels passing out from the diseased area (*d.w.v.*). $\times 7$. I = inoculum.



[FIG. 19. Disease record of a small and large inoculated decapitated Gros Michel root. $\times 2/3$. I = inoculum.

in the stele (Figs. 17 and 18); some of the wood-vessels passing out from the decayed tissue showed yellow and red discolourations typical of Panama Disease. These were the usual changes observed in this series of inoculations. One of the older and more mature roots, however, did not undergo this rapid die-back. The record is shown in Fig. 19. At the end of seven days the cut end showed only a small area of decay, from 0.3 to 0.6 cm., being more strongly marked on one side. The illustrations only show changes from Feb. 18, 1929, to Feb. 27, 1929, but altogether observations were continued till March 11, 1929, i.e. twenty-one days. The adjacent young root was killed off over its whole length in nine days. When

examined after twenty-one days all the lateral rootlets of the older root had become blackened and killed, but the terminal decay appeared to have been held in check. On examination of longitudinal sections it was found



FIGS. 20, 21, 22. 20. Longitudinal section through the end of the large root in Fig. 19. The fungus has only made a shallow penetration of the cortex, and has been held in check by the enlarged suberized cells, (S). The disease has made more progress in the stele, (st.). $\times 22$. 21. Transverse section through large root in Fig. 19, showing a diseased rootlet, r., passing through the cortex. Diffusion of toxic fungal secretions from the rootlet into the cortex has led to the formation of cambiform cells, with cell divisions at right angles to the direction of diffusion. Cambiform tissue, (c). Killing of cortical cells is being held in check by suberization of cell-walls. $\times 50$. 22. Another example of a diseased rootlet (r.) in the cortex. Note the well-developed cambium layer (c.), with strongly suberized walls (s.), towards the diseased rootlet tissue. Hyphae can be seen in the dead (stippled) cells at the top.

that while a certain amount of penetration along the stele had taken place the cortical cells had reacted in a significant manner to the presence of the fungus. In Fig. 17 it will be observed that the cells of the middle cortex have become distended and suberized, while the invasion of the stele is much slower than in the other roots examined.

An investigation of the diseased lateral rootlets was next made by cutting transverse sections through the main root. The infection established at the distal end of a rootlet gradually passes back into the cortex of the

parent root; the latter is thus liable to become infected. According to the toxicity of the fungal secretions diffusing out from the diseased rootlet cortical cells may be killed, when they acquire a dark-bluish colour, or they may be stimulated to react. In the latter case the cortical cells enlarge and divide by walls which are laid down parallel to the rootlet, i. e. at right angles to the direction of diffusion. When several such divisions have taken place the tissue produced has a cambiform appearance (Figs. 20 and 21). These structural changes are accompanied by the suberization of cell-walls, which is effective in preventing both the further diffusion of toxic substances and the penetration of hyphae. On the left-hand side (Fig. 20) two or three layers of cortical cells (adjacent to the rootlet) have been killed, but further away cells have enlarged, divided at right angles to the direction of diffusion of toxic secretions, and have become suberized. On the right-hand side, where the beginning of a cambiform formation is also seen, the extent of diffusion is more limited. This is shown in greater detail in Fig. 21. The dark cells on the outside have been killed and penetrated by the fungus, but further invasion is being held in check by well-marked suberization of adjoining walls. Lower down the cambiform cells, with strongly suberized outer walls, show no trace of hyphal penetration or death due to toxicity. To what extent infection is carried into the main stele by the rootlets remains to be seen. These observations show that in a root which has reached a certain stage of maturity rapid and complete killing does not take place, infection being resisted by marked structural and chemical changes.

V. RELATION OF SOIL ACIDITY AND ALKALINITY TO ROOT INFECTION.

In order to test a possible direct relationship between soil acidity and root infection the following experiment was carried out. Two pots each, of seven different soils, were completely sterilized in the autoclave, amply watered with sterilized water, and placed in 4,000 c.c. moist chambers containing about half-an-inch of water. The pots were then uniformly inoculated with a heavy spore suspension of *F. cubense* and left for several days. Suitable small suckers (sterilized externally with mercuric chloride solution and washed twice in sterilized water) from which roots were about to push out were then placed on the soil surface, one per pot (Fig. 23). The lids, unperforated, were then placed on, and the pots were left untouched for ten and twenty-six days. The soils selected gave a range of pH values from 5.8–8.1, while marked differences in chemical composition and soil texture were also provided. One of the soils used, a silt with pH 6.4, was taken from a notorious Panama Disease plot. A factor which remained constant throughout for each closed container was water-supply, as each pot was uniformly moistened at the outset and remained standing in an ample

reserve. After ten days one pot of each soil type was opened for examination. Varying amounts of root-growth had taken place, according to the nature of the soil and the sucker. On an average three roots per pot had developed. On careful examination of the roots and rootlets of each pot no single case of root disease, or even the slightest trace of infection, was found. The experiment was continued with the remaining pots for other sixteen days, i. e. twenty-six days in all, and at the end of that time, with

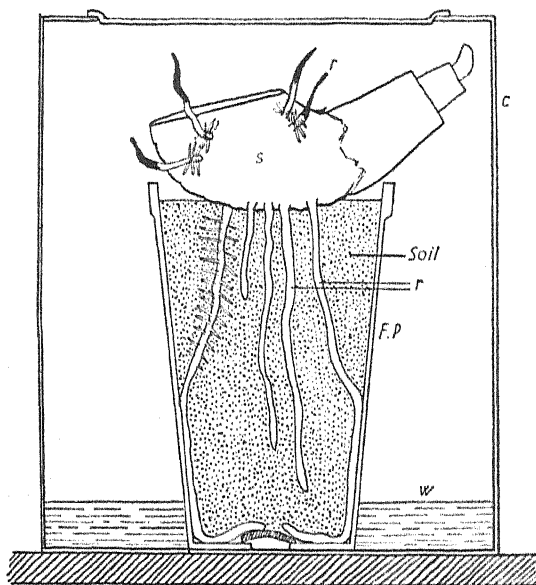


FIG. 23. Apparatus for testing root infection in different soils. C. = 4,000 c.c. container; W. = sterile water; S. = sucker; r. = roots; F.P. = flower-pot.

an average of four to five well-developed roots (with numerous rootlets) per pot, there was again no trace of infection. Roots which had grown out from the upper portion of the sucker into the air were diseased, and had suffered a characteristic die-back. This was attributed to the presence of the fungus, which had spread from the soil over the sucker, and to the concentration of CO_2 produced during the experiment. As the roots in the soil were not attacked, it would appear that the surface soil had acted as a buffer to the carbon dioxide.

This experiment, whose results have been confirmed by a large series of subsequent observations, indicates definitely that soil acidity, alkalinity, or mineral composition are not, of themselves, direct factors in promoting the attack of *F. cubense* on the roots of the Gros Michel. As accessory factors modifying the physical properties of soils under conditions of variable water-supply, however, they may be of the greatest importance.

VI. VARIABLE WATER-SUPPLY—A FACTOR IN ROOT INFECTION.

Suckers of uniform size were planted in ten-inch pots of soils similar to those used in Section V, the original idea being to determine whether any relation existed between root or sucker infection and pH value. Each pot, watered daily from above, rested in a pan of water to compensate for loss at the soil surface and for transpiration. The soils, which were not sterilized, were heavily inoculated with a mixture of several strains of *F. cubense*. When the pots were being turned out for examination of sucker infection a careful study of the roots was also made. Altogether some twenty pots were examined, thus bringing a total of more than 400 individual roots under observation. In spite of the heavy inoculations of *F. cubense* applied, the majority of the roots and rootlets were perfectly healthy and free from infection after periods varying from fifty to ninety days. Infections, however, were constantly found under two conditions irrespective of soil type. These were found (1) in the top inch of soil, which, though watered every morning, was subject to partial drying-out in the course of the day; (2) at the side of the pot extending about half-way down. Roots normally seek the side of the pot. When watered from above, drainage quickly takes place down the pot sides. While the lower pot sides are well supplied with capillary water from below, roots abutting on the upper part of the pot are subject to fluctuations in their water-supply, and also to a certain extent to heat-scorch. Their outer surfaces consequently show varying amounts of discolouration and disease.

Under these two conditions alone were root infections found. All other roots and rootlets grown under the favourable conditions of pot culture were free from disease. A typical partially diseased root from one of the pots might be described as consisting of the following segments: (a) healthy length of root extending horizontally from sucker to pot side through moist soil; (b) partially diseased root segment abutting on upper pot side, subject to fluctuations in water-supply; (c) healthy root segment growing down pot side in moist soil in lower half of pot; (d) healthy root segment growing in wet soil along bottom of pot.

That constant water-soak, as in (d) above, is not a condition leading directly to infection by *F. cubense* was clearly seen in these experiments.

In the top soil, subject to diurnal fluctuation in water-supply, root infection took various forms, and all stages from slight injury to complete killing were observed. Pot side infections were usually localized on one side of the root and complete killing was unusual. In most of the roots examined, as environmental conditions, with the exception of the variable factor in question, were favourable, marked reactions to injury and fungal invasions were observed.

VII. ANATOMY OF INFECTED ROOTS.

(a) *Slight apical infection.* Roots from the top layer of a pot of acid-clay soil (whose texture had been rendered open by the addition of 30 per cent. sand, pH = 5.8) showed slight local discolourations at the apex. Transverse sections revealed the first signs of fungal attack. Tissue only slightly affected showed a discoloured or collapsed piliferous layer. As

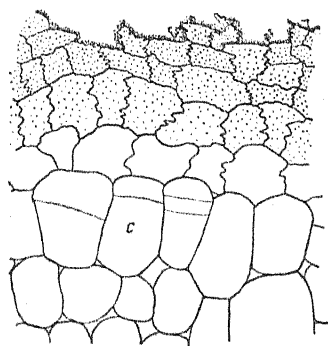


FIG. 24. Transverse section through apex of blemished Gros Michel root. The outermost tissues have been invaded by hyphae. A cambiform reaction (c.) to infection has commenced. Stippled cells have been killed by advancing fungal secretions. $\times 210$.

killing evidently takes place in advance of the hyphae by the diffusion of their toxic secretions, the innermost layers are progressively affected, and become dark in colour and plasmolysed, accompanied by corrugation of the cell-walls (Fig. 24). More deeply seated tissue reacts to the invasion, however, and cortical cells begin to enlarge and divide by tangential walls. Sometimes the reacting cells are adversely affected by toxic substances before they have become suberized. There is thus a race between the diffusion of killing substances arising from the fungal metabolism and the formation of a layer of protective tissue. Inside the cambiform layer cells may also have been stimulated to increase in size. Once the suberized

layer is formed all further invasion is checked; hyphae were never observed to pass inside, and the hyaline appearance of the cells indicated that the lethal substance had not diffused through the suberin barrier. The slender hyphae of *F. cubense* were constantly associated with the outer tissues, but penetration appeared to be slow. In slightly older portions of the root, where blemishes were more marked, the layer of penetrated dead outer tissue, six to eight cells deep, was bounded within by a double layer of enlarged cells with suberized outer walls. Hyphae were localized outside the barrier of hypertrophied tissue.

(b) *Constricted roots.* Some of the roots from top soil showed brownish-yellow colour and constricted zones quarter to half-an-inch in length. They were otherwise turgid and healthy. From transverse sections it was seen that the outer tissues of the constricted zones had been invaded and killed to a depth of four or five cell-layers. Beyond this there was no further invasion, because of the presence of a well-developed cambiform layer with suberized outer walls. This formation, extending right round the root parallel to the periphery, had originated by the radial elongation and subsequent tangential divisions of a single layer of cortical cells, so that a definite cambiform tissue, regular in appearance and six to eight cells

deep, was formed. Suberin had been deposited on the outer walls and also for a short distance along the radial walls, and sometimes on the first and second tangential walls. The hyphae of *F. cubense* and sometimes of other fungi were found in the dead outer tissue right up to the cambiform layer, but beyond this there was no further penetration. This protective mechanism developed in roots which have sustained a physiological shock is definitely effective in preventing the passage of parasitic fungi into the vascular system. The strong reactive power of healthy roots is clearly indicated by these structural and biochemical changes.

(c) *Deep lesions at soil level.* Some blemished roots from top soil were found to have deep lesions, about one-eighth of an inch in length. A representative section is shown in Fig. 25. The outer tissues had been invaded, killed, and disintegrated. Cells so affected developed an inky-black appearance, and invading hyphae could be discerned. In the region of the middle cortex, however, healthy tissue had reacted to the invasion stimulus, and a well-marked cambial formation had resulted. Evidently there had been several centres of deeper invasion, and the distension of the outer tissue by the growth activities within had led to the formation of the deep slit-like lesions. The cambiform layer, some six to seven cells deep, was suberized on the outer side, and had not been killed by fungal secretions or penetrated by hyphae. Outside the suberized layer there was evidence of arrested cambiform formations which had been overtaken by lethal secretions before suberin was deposited in their walls. The far-reaching effect of such fungal secretions is further indicated in Fig. 25, where cortical cells abutting on the endodermis have been stimulated to radial expansion.

(d) *Severe infections.* In sandy soils of open texture it was frequently found that top-soil roots were limp and discoloured over a distance of an inch to an inch and a half. When placed in water they quickly acquired a water-soaked appearance. Such roots were found to be badly diseased, with abundant hyphae in the cortex and wood-vessels, and with characteristic Panama Disease red and yellow colours spreading along the stele from the centre of mass infection. In such infections, resulting presumably from severe drought, no protective mechanisms were found to be in operation.

(e) *Apical die-back of roots.* Under the previous heading a highly diseased condition of adult roots in top soil was described. In some pots a similar type of severe infection, affecting the apex and causing a gradual die-back, was found. In one pot (potting compost) six roots with apical die-back were found in the top inch of soil, while in another (an alkaline soil with sand) five were found. The physiological shock sustained evidently was such as to make for deep fungal penetration, with the result that both cortical and stelar tissues were invaded and exploited. The slow dying-back which ensued was accompanied by the appearance of yellow and red colours in the wood-vessels. Longitudinal sections did not reveal

any protective modifications adjacent to infected tissues. The open middle cortex was exploited to a greater extent than the more compact outer or inner cortical tissues. The death of cells was accompanied by a considerable amount of gum secretion, the latter being responsible for much of the dark coloration in stelar parenchyma.

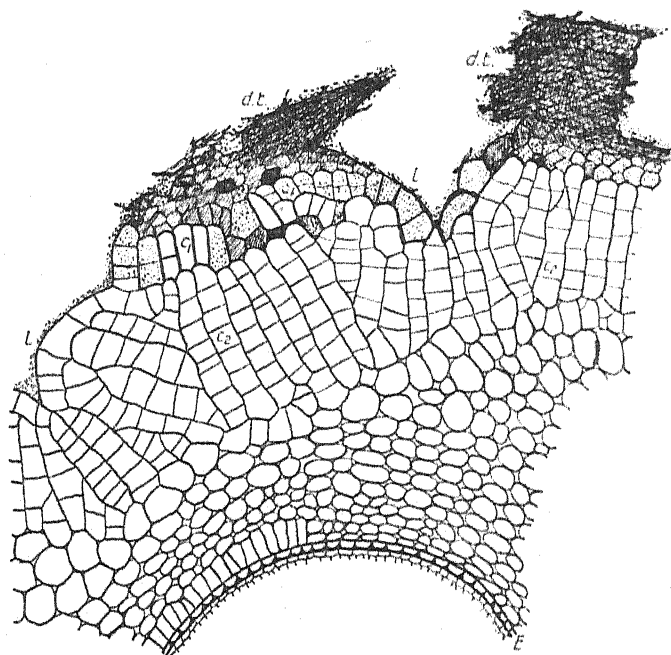
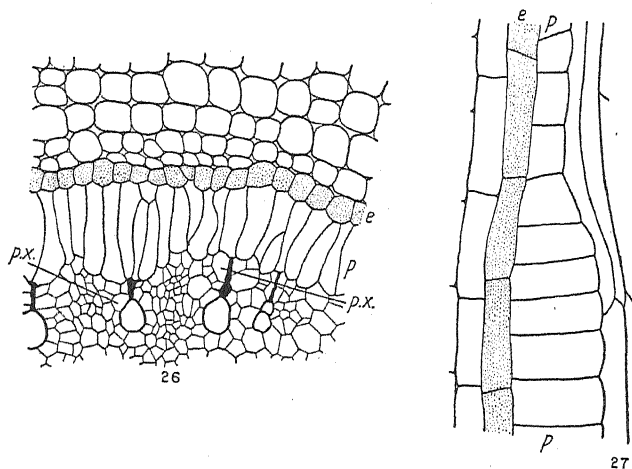


FIG. 25. Transverse section of a Gros Michel root with deep lesions (L.). Outside are zones of invaded and decaying tissue (d.t.) and regions of attempted formation of a cambiform barrier (C₁). Many of the latter cells, however, have been killed, but further invasion is definitely held in check by the cambiform tissue (C₂). Enlarging cells are also seen just outside the endodermis (E.). $\times 70$.

The absence of defensive mechanisms was noted in most of these instances of apical die-back examined. In one badly diseased tip, however, a growth reaction was found in the pericycle. In transverse section the latter was found to have expanded considerably in a radial direction, but there were no accompanying tangential divisions. The abutting protoxylem was shrivelled, whilst the central stelar parenchyma was stained and gummy (Figs. 26 and 27).

(f) *Partial disease of rootlets.* While suberized cambiform layers may prevent deep penetration of the cortex and stele, the latter may still be infected by way of diseased rootlets traversing the cortex. Examples of such diseased rootlets have already been cited: see Figs. 21 and 22. During the course of these investigations it has frequently been observed that while rootlets, because of their more delicate nature, may be diseased

and shrivelled, the main stele is not necessarily infected. Some additional information may now be adduced. A semi-aerial root, which had gradually pushed itself out of inoculated soil, had numerous rootlets which were



FIGS. 26 and 27. Growth reaction in pericycle of diseased root-tip in transverse section and longitudinal section—(p.x.) collapsed protoxylem, (p.) pericycle, (e.) endodermis. $\times 210$.

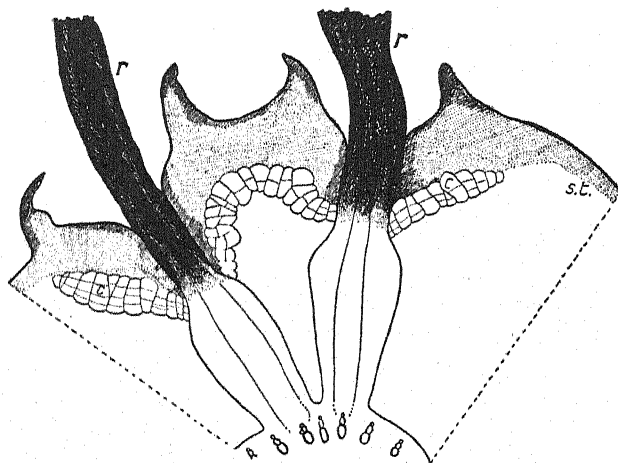


FIG. 28. Transverse section of a healthy Gros Michel root with diseased rootlets (see text). The inward dying back of the rootlets (r.) has stopped at the cambiform layer (c.); (s.t.) suberized tissue. $\times 30$.

shrivelled and diseased. The main root, of a corky yellowish-brown colour, was turgid and healthy. In transverse section it was found that the outermost layers of cells were suberized. Where rootlets passed out there were local centres of infection, the more peripheral tissue being invaded by hyphae. This infection, however, was cut off by the formation of a well-developed suberized cambiform layer (Fig. 28). The peculiar point which

was observed, and for which a full explanation cannot yet be given, was that the diseased condition of the rootlets did not extend inwards beyond the cambiform barrier. The main root was sectioned over its whole length, thereby exposing many rootlets, and this was found to hold good throughout. There was thus no infection of the main stele.

(g) *Root infection at pot side.* As explained in an earlier section, roots were constantly found in a blemished or diseased state along the side

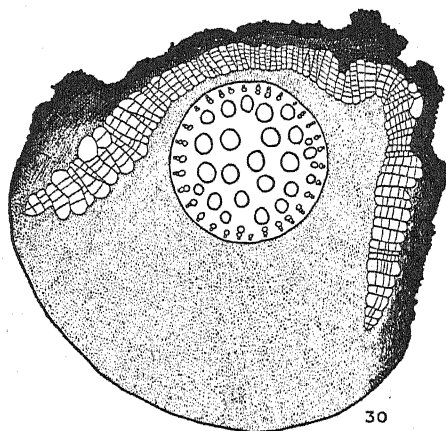
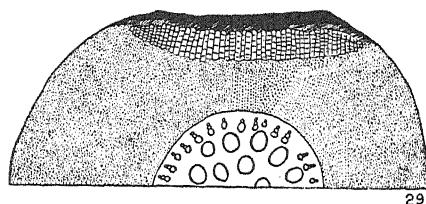


FIG. 29. Diseased Gros Michel root, with a peripheral protective cambiform layer. $\times 32$.
FIG. 30. A more deep-seated invasion, with a well-developed protective cambiform layer. $\times 32$.

of the pot where drainage is rapid and where upward movement of water by capillary action is apparently not effective. Under these conditions of variable moisture supply, and possibly heat-scorch, all stages from shallow to deep infection were found. As roots are normally in a high state of virility under conditions of pot culture, however, it was constantly found that a well-developed suberized cambiform barrier had been formed. Diseased roots were readily recognized by dark superficial discolorations. Where infection was slight, disease was localized on the side lying against the pot side. A typical section is shown diagrammatically in Fig. 29, where the peripheral tissue has been invaded and discoloured; deeper penetration and subsequent stelar infection have been definitely prevented by the formation of a crescentic suberized cambiform layer of tissue, some

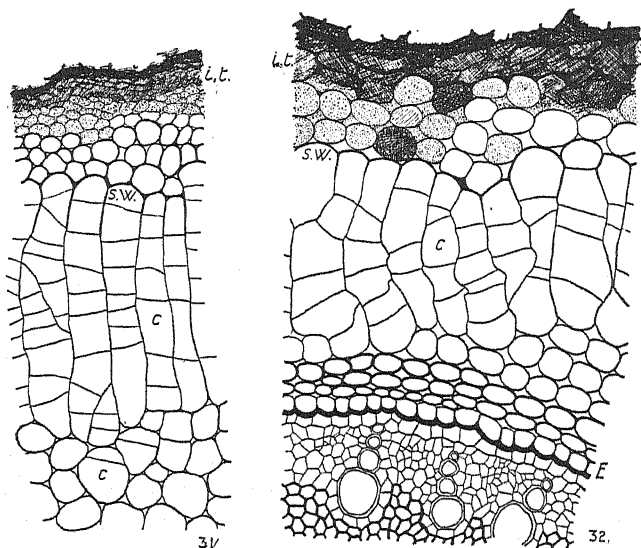
six cells deep. More detailed observations are shown in Fig. 31. Three diseased zones can be observed; (a) the outermost layer of cells, disintegrating after complete exploitation; (b) layers of cells invested by hyphae; (c) layers of cells which have been killed in advance by toxic fungal secretions; these are usually of a dark bluish-black colour, but no hyphae are present. Further in are cells which have reacted to fungal stimulation by partial or complete suberization, and still further in are cortical cells which, by considerable radial expansion and tangential septation, have formed the well-defined cambiform layer. Their outer walls are strongly suberized. The presence of suberin was demonstrated by direct microscopic observation in a water mount, by the non-absorption of water-soluble stains, by colour reactions with chlor-zinc iodide and with potash solution, and by its insolubility in concentrated sulphuric acid (4). When sections were placed in the latter, all the cellulose dissolved away, leaving behind the suberin skeleton. The radial walls were suberized about one cell deep, and sometimes this extended also to the first and second transverse walls. In some sections it was found that the fungus had invaded cells right up to the cambiform barrier, but in no case was deeper penetration observed.

That any normal, young and unspecialized cortical cell may take part in the formation of cambiform tissue was demonstrated in roots where infection was more deep-seated (Fig. 30). It will be seen that, while at some points the infection is peripheral, as in Fig. 29, at others the cortex has been deeply invaded, and a layer of inner cortical cells, some three or four layers removed from the endodermis, has been responsible for the formation of the cambiform barrier. Thus, although deep invasion of the cortex may take place, the stele still remains unpenetrated by hyphae (Fig. 32).

(h) *Infection through old root bases.* Before planting, all the roots are trimmed off close to the sucker. As *F. cubense* is essentially a wound parasite, these exposed root bases would appear to offer points of easy access to the sucker. A considerable number of such root bases, from suckers growing in inoculated soils for periods of 70 to 90 days—an ample time for effective penetration—were examined. The suckers were growing under favourable conditions of soil moisture and aeration. In practically all cases it was found that no effective parasitism had resulted from penetration through these possible points of entry.

Where the diseased root base abutted on the tissue of the sucker the latter had developed a characteristic cambiform layer with suberized walls at right angles to the direction of diffusion from the root. The partial penetration of the stele in the root base will be subject of further investigation; the large number of cut roots where significant penetration does not take place suggests that there is some mechanism, structural or biochemical, by which the longitudinal invasion of the stele is prevented. In many

vessels it was found that gum deposits and tyloses were of frequent occurrence. According to Haberlandt (5): 'The functions of tyloses seem to be somewhat variable. They occur with special frequency beneath the scars of branches that have broken off, and also near the severed ends of cuttings, being in both cases developed in such numbers that they become flattened by mutual pressure, and completely fill the cavities of the vessels for a considerable distance; in these instances it can hardly be doubted that they



FIGS. 31 and 32. 31. Transverse section of a diseased Gros Michel root, showing peripheral infection. $\times 90$. 32. Transverse section showing a deep-seated infection. $\times 210$. (i.t.) tissue invaded and exploited by hyphae; (s.w.) protective suberized walls of cambiform formation (c.); stippled tissue has been killed in advance by toxic secretions. (E.) endodermis.

serve, as Boehm first suggested, to plug the cavities of the conducting tubes that have suffered mechanical injury. The tyloses which appear in heart-wood, or in the older portions of splint-wood, in the absence of an injury, apparently fulfil a similar purpose.'

The observations so far made on root bases suggest that the formation of tyloses may prove to be important in preventing the passage of hyphae along the wood-vessels leading to the sucker stele.

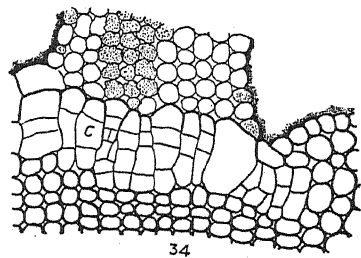
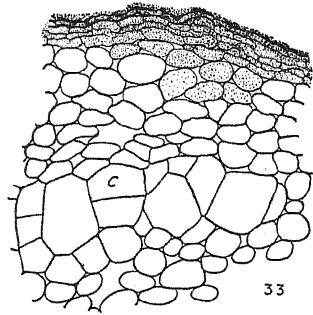
VIII. ROOT INFECTION OF *MUSA CAVENDISHII*. (CANARY BANANA.)

While detailed discussion of root disease in the highly resistant (or immune) Canary banana (*Musa cavendishii*) must be held over till a little later time, some observations germane to the subject of infection-reaction may, with advantage, be discussed briefly at this point. It was found, for example, that a slight infection could be induced in roots growing in unventilated moist chambers. While the roots were not severely affected

sections showed that penetration of the superficial cells had taken place, and that a cortical hypertrophy had resulted as in the case of the Gros Michel.

Surface roots, subject to periodic drought, of a plant grown in a tub of acid-clay soil were found to be blemished and diseased. The diseased areas were superficially ovoid, or oblong, and of bluish colour, while more severely affected roots showed marked loss of turgor. Whether the infections were due to *F. cubense* alone, or to other soil organisms present, could not be determined, as unsterilized soil was used. The important point which emerged from detailed anatomical observations was that this species also shows tissue reactions comparable to those described for the Gros Michel. An early stage of infection is shown in Fig. 33 and an advanced one in Fig. 34.

In the former the outer tissues have been invaded by fungal hyphae, killing in advance has taken place, and at some distance further in healthy cortical cells have commenced to expand and divide tangentially. In Fig. 34 a very deep infection is shown. All the outer cortex has been invaded, ex-



FIGS. 33 and 34. Cambiform tissue formation (c.) in the roots of the Canary banana, *Musa cavendishii*. 33. A peripheral infection in an early stage. $\times 210$. 34. A deep-seated infection in an advanced stage ($\times 90$).

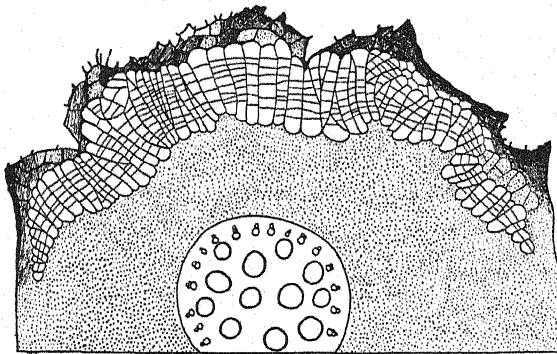


FIG. 35. Superficial lesions on the roots of *Musa cavendishii*. Fungal invasion has been checked by the formation of a well-defined suberized cambiform layer.

ploited, and disintegrated. The inward progress of the disease, however, has been checked by the formation of a well-defined cambiform layer with suberized outer walls. Diseased tissue was frequently observed to be sloughed

off along the line of suberization. Again, while hyphae could be observed outside the cambiform layer, they were never observed to pass through it, nor were its component cells seen in a state of collapse or decay. This brief reference to the roots of *Musa cavendishii* indicates that it agrees with *M. sapientum* in its general reactions to parasitic invasions.

IX. DISCUSSION.

Drost (3), writing of Panama Disease in Surinam in 1912, remarked that the disease was most apparent about the end of the rainy season and the beginning of the dry period. He further remarked, *a propos* of estates where no disease had occurred, and where topography made for favourable soil moisture relations: 'The soil remained moister in consequence, and the influence of the long dry season was blunted. Dryness, and especially very wet and very dry soil conditions succeeding each other rapidly, favour the disease strongly', and again: 'A varying humidity of the soil increases the susceptibility of the banana to disease, while a uniformly moist soil reduces it.'

Brandes, writing of Porto Rico and Hansford of Jamaica, remarked that there is a decided increase in the number of apparently new cases of disease shortly after the wet season has set in. Without trying to reconcile the many differences in statements regarding the relation of climate to disease, it can be said with safety that disease is definitely related to those periods when variable soil moisture is the rule. This contention is further borne out by the fact that little disease has been reported from arid regions where irrigation (i. e. controlled soil moisture) is the rule.

In seeking to discover and assess the extent of possible external factors, the writer has so far confined himself to a detailed study of controlled experiments in greenhouse and laboratory. The results show that the roots of the Gros Michel banana, when maintained under suitable uniform conditions, do not readily succumb to the parasitic activities of *F. cubense*. It has further been demonstrated that soil acidity, alkalinity, or chemical composition, are not, of themselves, direct factors promoting parasitic attack. The two conditions under which positive infections were obtained were (1) carbon dioxide vitiation and (2) variable soil moisture. While exact measurements of these two factors have not yet been made, their importance has been indicated by the observations submitted. The relation which they bear to two of the major problems of untilled virgin tropical soils, namely, aeration and water supply, has been commented on in the Introduction, while their significance with regard to the broad ecology of the disease in Central America and the West Indies has been sufficiently discussed in recent travel reports (9, 14). In the detailed observations submitted here, it has been shown that the degree of infection

varies from superficial blemishing to complete rotting. A factor which can only be described in general terms, namely, virility of individual roots, has been shown to be of considerable importance. That the fungus does not parasitize roots under all conditions has been amply demonstrated. Further, it has been shown that where root tissue is still unspecialized and in a state of health, biochemical and structural changes take place, whereby both hyphal penetration and diffusion of toxic substances are completely held in check. When conditions permit the fungus to occupy the whole transverse plane of the root, however, a gradual die-back inevitably takes place.

The formation of cambiform tissue has been demonstrated in all parts of the root cortex of both *M. sapientum* and *M. cavendishii*. Its position bears a definite relation to the direction of diffusion of toxic fungal secretions, i. e. at right angles. Thus, while the cambiform bands are usually disposed parallel to the circumference, it has been shown that they may also be formed radially, i. e. parallel to a diseased rootlet from which diffusion of toxic substances is taking place. They may also be disposed transversely across the cortex in relation to longitudinal invasion. The presence of suberin in the outer walls of the cambiform cells has been shown to act as a definite impediment to diffusing toxic substances and to hyphal penetration. The discovery of these tissue reactions is regarded as important because of the useful indication they give, not only of the many changes brought about in roots by the presence of a parasitic organism, but also of the physiological state of the root tissue itself.

In the author's opinion, provisionally stated, root infection is much less important than sucker infection, except where it takes place in proximity to the root-base when sucker infection may result. The latter is regarded as the fundamental problem in considering the production of the major wilt symptoms.

X. SUMMARY.

1. A brief summary of Panama Disease literature is given. It is a wilt disease of great severity, caused by the parasitic soil organism, *F. cubense*, on suckers and roots of the Gros Michel banana.
2. While the susceptibility of this variety has long been known, no critical experiments have been carried out to determine if this is constant for all external conditions.
3. Inoculated, healthy roots, grown in ventilated moist chambers, did not become infected. Smaller, less virile, roots became slightly infected.
4. Roots growing in unventilated moist chambers, when inoculated at the apex, showed a cessation of growth, partial infection, discolouration, and production of basal rootlets. The latter readily became diseased.
5. Several cases of inconclusive inoculations are discussed.

6. Young decapitated roots on inoculation showed a rapid dying-back. Older roots resisted penetration to some extent by structural and biochemical reactions.

7. It was shown by experiment that soil acidity, alkalinity, or chemical composition are not, of themselves, direct factors promoting infection.

8. Variable water-supply was shown to be an important factor in root infection. The latter varies from superficial blemishing to complete killing.

9. Healthy roots which have become partially diseased show marked biochemical and structural reactions. Protective suberized cambiform layers develop in the cortex whereby further hyphal penetration and diffusion of toxic secretions are prevented.

10. A general discussion of results is appended.

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NOTE.

NEW LIGHT ON THE SO-CALLED PARASITISM OF *ACTINOCOCCUS AGGREGATUS*, Kütz, AND *STERROCOLAX DECIPIENS*, Schmitz.—*Gymnogongrus griffithsiae*, Mart., occurs in the highest of the rock-pools at Aberystwyth, and all except the youngest plants bear nemathecia of the so-called parasite *Actinococcus aggregatus*, Kütz. *Ahnfeltia plicata*, Fries, occurs abundantly in the deeper rock-pools, and a large percentage of the plants bear, particularly during the winter months, a heavy infection of the so-called parasite *Sterrocolax decipiens*, Schmitz.

No reproductive organs have previously been recorded for either *Gymnogongrus griffithsiae*, Mart. or *Ahnfeltia plicata*, Fries, and only the asexual phase of the so-called parasites is known, *Sterrocolax decipiens* reproducing by monospores, whilst from the nemathecia of *Actinococcus aggregatus* both monospores and tetraspores are produced.

Although *Actinococcus* and *Sterrocolax* have been described by former workers as internal parasites, yet in no case have stages of entry been observed. Wherever the pustule-like thallus occurs, it shows connexion with the so-called 'host' by 'penetration-filaments'. A study of developmental stages indicates, however, that the pustule is developed from within outwards and that it is in no sense an external parasite.

Serial sections through the stipe of *Gymnogongrus griffithsiae*, in the neighbourhood of an *Actinococcus* nemathecium, have revealed the presence of what appear to be rudimentary procarps. Such structures exhibit variety of form but typically consist of an enlarged bearing cell surmounted by a two-celled carpogonial branch. Occasionally there are indications of a trichogyne.

The bearing cell ultimately assumes a stellate form, extending outwards into arm-like protuberances, from the ends of which cell-filaments are produced. Some of these filaments penetrate between the cells of the limiting layer and emerge as sub-cuticular 'pockets' of tissue. Masses of cell-filaments occurring between the medullary cells of *Gymnogongrus* may originate in a similar manner.

It has been established that the 'pockets' of tissue finally penetrate the cuticle of *Gymnogongrus* and contribute to the extramatrix tissue of *Actinococcus*. Male organs of *Gymnogongrus griffithsiae* have not been found, and it has not been ascertained whether fertilization takes place.

An attempt has also been made to trace the origin of the nemathecial filaments of *Sterrocolax decipiens*. It was found that, prior to any visible sign of the cushion-forming parasite, a localized hypertrophy of the cortical tissues of the so-called host plant takes place. This has nothing to do with the secondary growth which appears when the plants are exposed to rough seas.

Within such a tissue cell-filaments occur, terminated by darkly-staining, somewhat pointed apices, and it is believed that these filaments give rise to the extra-matrix

tissue of *Sterrocolax decipiens*, the apical cells pushing their way between the linear series of cells of the hypertrophied cortex and collecting in small darkly-staining masses beneath the cuticle of *Ahnfeltia*.

Such outgrowths eventually become confluent with one another, and later form the so-called nemathecium of *Sterrocolax decipiens*. At this stage there is practically no sign of the darkly-staining apices within the hypertrophied cortex.

The origin of these filaments could not be traced, but they appeared to be continuous with the outer medullary cells of *Ahnfeltia plicata*.

In the neighbourhood of such infected areas very definite fusions occur between some of the medullary cells of *Ahnfeltia*.

These fusions are frequently seen to be preceded by alterations in the composition of the cell-walls and by the highly granular condition of the cell protoplasm.

Fusions between the medullary cells do not take place merely by the enlargement of pit-connexions but also by the formation of definite outgrowths between neighbouring cells.

The prevalence of this phenomenon in infected regions appears to be significant, and it is possible that the fusions between the vegetative cells might represent a very much reduced sexuality.

The later more elaborate fusions formed by the enlarging of pit-connexions and disintegration of intervening walls might correspond with the secondary fusions which normally take place in a Red Alga between the sporogenous filaments and other vegetative cells for the nutrition of the carposporophyte.

A few mature *Ahnfeltia* plants, occurring in close proximity to other heavily 'infected' plants, bore no signs of *Sterrocolax decipiens*. It is possible that they are male plants although male reproductive organs were not observed.

Monospores are liberated from the nemathecium of *Sterrocolax decipiens*, whilst in the nemathecium of *Actinococcus aggregatus* chains of tetraspores are occasionally produced, although as a rule the tetrad division is incomplete and only monospores are formed.

The monospores of both so-called parasites have been grown in culture, being supplied with nutrient solutions containing either sodium or potassium salts. Their rate of growth, however, is extremely slow, and after three to five months, only rudimentary disc-like structures have been obtained.

A preliminary cytological investigation has been carried out. The medullary cells of *Gymnogongrus* contain several very small nuclei, and there is some evidence that they have four chromosomes.

The potential monosporangia of *Actinococcus aggregatus* appear to have either four or eight chromosomes, but chromosome-counts of the tetraspores have not yet been obtained.

The medullary cells of *Ahnfeltia plicata* exhibit both a four and an eight chromosome complex in their nuclei, but it is not yet known whether the eight-chromosome condition bears any relation to the cell-fusions. There is some evidence that there are eight chromosomes in the apical cells and in the monospores of *Sterrocolax decipiens*.

Actinococcus aggregatus, Kütz., thus appears to be the asexual biont of *Gymno-*

gongrus griffithsia, Mart. No male organs have been found, and in many cases the female reproductive organs appear to be degenerate.

The mode of origin of *Sterrocolax decipiens*, Schmitz, and its similarity of structure with *Ahnfeltia plicata*, Fries, suggest the probability that this too is the asexual biont of its so-called 'host'. Conclusive results from a cytological investigation and from the culture of the asexual spores have not yet been obtained.

From these results it would appear that the life-histories of *Gymnogongrus griffithsia*, Mart., and *Ahnfeltia plicata*, Fries, can be correlated with that of *Phyllophora Brodiae* as recently elucidated by Rosenvinge. ('*Phyllophora Brodiae* and *Actinococcus subcutaneus*.' Der. Kgl. Danske Videnskabernes Selskab. Biologiske Meddelelse, viii, 4.)

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The Relationship of *Garrya*.

The Development of the Flowers and Seeds of *Garrya* and its Bearing on the Phylogenetic Position of the Genus.¹

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With Plate XXXVI and forty-nine Figures in the Text.

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I. INTRODUCTION.

1. *Nature of the Problem.*

THE position of *Garrya* among the Dicotyledons has, for practically a century, been the subject of a dispute which has not as yet terminated in any measure of agreement. Moreover, the opinions of the different authors who have written of the structure and relationship of *Garrya* have been curiously diverse. Few seed plants have had for so long such an unsettled taxonomic status. The plant has been allied by many botanists with the highest Archichlamydeae, by some with the primitive Dicotyledons, while various other taxonomists have assigned it to one or other intermediate position. This diversity of views is due in part to the contradictory nature of the various reports on the structure of *Garrya*—that is, to the still

¹ Botanical Contribution from the Johns Hopkins University, No. 100.

incomplete and uncertain knowledge of many features of its structure that must be definitely known before this genus can be properly classified.

Garrya elliptica was discovered by David Douglas, of London, during his second expedition to Western America in 1826, and named by him after Nicholas Garry, of the Hudson Bay Company, who aided him in his expeditions. Douglas carried to England specimens and seeds of *Garrya*, and eight years later (1834) staminate plants blossomed in London, arousing, by their curious nature, the intense interest of English botanists. Lindley at this time published the first detailed description of *Garrya*, deriving his information concerning the pistillate plant from the herbarium specimens collected by Douglas. In this report he described the flowers of both sorts as arranged in catkins or aments—three flowers behind each of the opposite, connate, decussate bracts. The four perianth parts of the staminate flower he called sepals. The ovary is described as inferior, surmounted by a bidentate calyx and two styles, and as containing two ovules, each pendent from a lateral placenta. The ovule is described as consisting of a minute embryo lodged in a fleshy, homogeneous albumen, surrounded by two testae, the outer thin and membranous, the inner thin and transversely ridged. The germination of the seed is reported as 'intra-seminal', the hypocotyl emerging first, the plumule then arching out between the two cotyledons, while these latter remain permanently anchored in the seed.

This publication of Lindley's formed the essential basis for all descriptions of *Garrya* that appeared during the next forty years. The conclusions based on it, however, differed widely. Lindley himself held that the floral structure of this genus is entirely different from that found in any known order of Dicotyledons. He, therefore, constituted for it the family Garryaceae, and even a new natural order, the *Garryales*, which he referred to the 'Urtical Alliance'. Later he changed this opinion and placed the *Garryales* in the 'Euphorbial Alliance'. Endlicher considered this plant as belonging to the *Apetalae*, and placed it under the 'Antidesmeae'. Agardh classed it under the Amentaceae in a sub-order co-ordinate with the 'Cupuliferae'. Both Brongniart and Jussieu placed it provisionally, though with serious doubts, under the Cornaceae. Bentham and Hooker, however, included it definitely in the family Cornaceae. Decaisne endorsed this conclusion in 1873, but later removed *Garrya* from the Cornaceae and placed it under the Hamamelidaceae.

In 1877, Baillon began his memorable research on the living flowers of *Garrya*, both staminate and pistillate, which were then blossoming in the Jardin des Plantes at Paris. In many respects his findings differed markedly from those of his predecessors. He was the first to interpret the perianth members of the staminate flower as petals, stating among his reasons that in its early development the flower shows, not only the primordia of these organs, but also a second circle of four papillae surrounding and

alternating with the first. These external papillae he considered to be the rudiments of true sepals. The organs of the pistillate flower, which had been called sepals by earlier writers, Baillon interpreted as 'bracts drawn up to midway of the ovary, rarely at the top as true perianth parts should be'. He reported the ovary as 'not infrequently crowned by a floral envelope surrounding the base of the styles, the divisions of which alternate with the bracts mentioned above, and are sometimes well developed and petal-like in appearance'. He concluded, however, that the irregularity of the occurrence of this would indicate it to be some sort of morbid hypertrophy, such as might follow the sting of an insect.

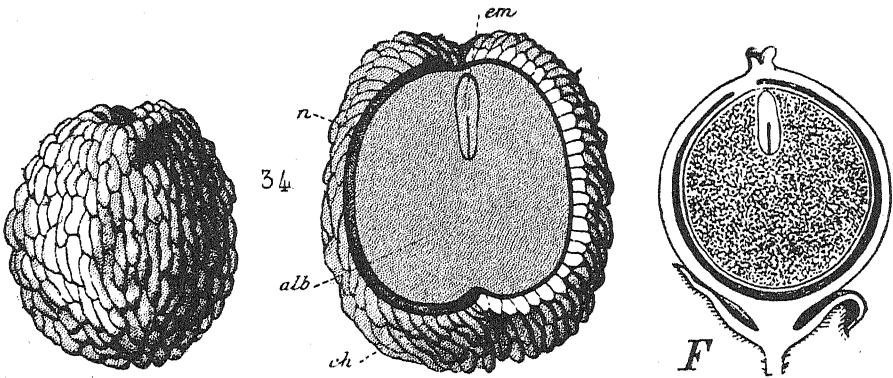
Baillon regarded the ovary as superior, and supported this view by the statement that many staminate flowers possess a hollow rudimentary gynaecium consisting of two carpellary leaves projecting above the bases of the four stamens. His description of the ovary shows further differences. In his first contribution he interprets the fruit as that of one of the Cornaceae in which the placentae have not developed sufficiently to divide the cavity into two locules. In a later paper, however, he reports the occasional occurrence of ovaries with three stigmas and a corresponding number of carpels. In his first paper he describes the fruit as follows: 'The fruit is little known. The pericarp is thin and almost completely dry at maturity. It is a membrane then without sap. Nevertheless, there is something of flesh and pulp in the fruit. It furnishes one of the rare examples known as an "arille generalisé". During maturation the superficial layer of the integument swells in all parts. The cells radiate as a sort of foam of viscous liquid (the development is very rapid); their appearance suggests the strophioles of certain Papaveraceae. At first they are almost colourless, but later they become wine purple, acid, and slightly bitter to the taste. They finally come to apply themselves to the pericarp, the thickness of which remains inconsiderable. The fleshy portion of the fruit comes from the integument of the seed.'

The illustrations accompanying this description (Text-figs. 1, 2, p. 774) show a subglucose body enveloped in a thick layer of short plump cells which are longest in meridional direction.

In spite of this detailed description, given in his first paper, Baillon seems to have been subject to a gradually increasing doubt of his observations. In his second contribution this feature received brief mention; in the third the description is relegated to a note, and in the fourth it is omitted entirely. For his earlier drawing is substituted, in the two later papers, a figure showing a minute embryo lodged in the large endosperm and surrounded by a thin integument and a thin ovary wall, represented by a single line (Text-fig. 3). This drawing became a classic, and is the one almost invariably reproduced in subsequent reports on the morphology of *Garrya*. In his fourth and last paper on *Garrya*, Baillon (5) speaks of

the integument as being 'very simple and incomplete, as is that of many of the Cornaceae'.

The classification of *Garrya* under the family Cornaceae entirely satisfied the judgement of Baillon, and, as a confirmation, he reported



TEXT-FIG. 1.

TEXT-FIG. 2.

TEXT-FIG. 3.

TEXT-FIGS. 1-3. (For explanation see text.)

marked success in reciprocally grafting *Garrya* and members of the Cornaceae (especially *Aucuba japonica*) upon each other. These observations and conclusions of Baillon's largely superseded those of Lindley as a basis for later discussions. Warming (1895), adopts the same classification, while Bonnier and Du Sablon (1901) place the Garryaceae between the Hamamelidaceae and the Saxifragaceae.

Harms (25) rejected Baillon's report of a rudimentary gynaecium in the staminate flower, but accepted his interpretation of the perianth parts. However, like the earlier workers, he considered the 'follicles' on the ovary as representing a bidentate calyx. He agrees with Baillon's last report of the testae, describing them as thin and membranous. Unlike Baillon, he regards the placing of *Garrya* under the Cornaceae as very unsatisfactory, but since many essential points of structure were still unknown attempts no reclassification.

Wangerin (1907), in his detailed discussion of the Cornaceae, noted the presence of a rudimentary gynaecium in the staminate flower, 'almost without exception'. It was said to be commonly very small, but sometimes comparatively large, and to show in both horizontal and vertical sections a distinct ovarian cavity, destitute of ovules, however. He argues at length in behalf of Baillon's interpretation of the 'folioles' on the ovary, vigorously disputing the opinion of Harms and the earlier workers. He confirms Baillon's final report of the thin membranous testae, at the same time quoting the latter's earlier description of an 'arille generalisé'. On the

other hand, he rejects Baillon's classification of *Garrya* with the Cornaceae, and in 1906 lists it under the Amentaceae. In 'Das Pflanzenreich', however, Wangerin (45) follows the conservative policy of Engler, and classifies it under the Cornaceae as an exceptionally aberrant form. Engler and Gilg (20) again regard *Garrya* as representative of a distinct order, which is, however, placed among the most primitive Dicotyledons, preceded only by the orders *Verticillatae*, *Piperales*, and *Salicales*.

According to Hutchinson (28) the *Umbelliferae*, as well as a number of other orders, are polyphyletic—that is, the arborescent and herbaceous members of certain orders have arisen through different lines of ancestors. In Hutchinson's classification the *Garryales* are removed from the *Umbelliflorae* and placed with the *Salicales*, *Fagales*, a part of *Urticales*, &c., as members of the group 'Amentiferae'. This classification, however, is based entirely on the descriptions of older writers discussed above. It differs from the others because of Hutchinson's different interpretation of the facts they recorded.

In general it may be stated that wherever *Garrya* has been placed in a family or order with other plants, this has been done always with hesitation, and it has been regarded as an aberrant member of that group.

Meanwhile, in America, the native home of *Garrya*, the botanists who are interested in this genus have concerned themselves chiefly with reporting newly discovered species, with arranging these species within the genus, and with eliminating duplicates. This task also is the subject of controversy since, as Miss Eastwood shows, the inaccessible habitat of the genus and the unusual blooming and fruiting seasons have prevented the collection of a complete representation of the developmental stages of each species in the different herbaria of the country. Since the flowers and the fruits are so little known, such external features as size of plant, size, shape, and character of edge, and pubescence of leaves form the essential division of the genus into species. Miss Eastwood (17), for example, distinguishes the species primarily by the branching of the 'aments'; secondarily by the nature of the hairs composing the tomentum. Notable among such contributions are those of Torrey, Bolander, Brewer and Watson, Coulter and Evans, and Miss Eastwood. These writers, as a rule, recognized the phylogenetic problem presented by *Garrya*, but made no attempts at demonstrating its relation to other Dicotyledons. Their attitude is well indicated by the following words of Coulter and Evans (13, p. 93): 'The three genera of this order (Cornaceae) represented in North America are so different from each other that they have often been separated into as many orders. Our purpose, however, is not to discuss their ordinal relationships but merely to present their species. For this reason we give no generic descriptions but accept the genera as ordinarily understood.' These authors, in referring to *Garrya*, say: 'This peculiar south-western

and Mexican genus seems to bear no resemblance to our other genera of Cornaceae.'

2. *Present Investigation.*

Hofmeister (1869-61), Hegelmaier (1864), and many others, including quite recently Shürhoff (39), have convincingly shown that a knowledge of sporogenesis, together with the development of the seed, embryo sac, and embryo, are often of great importance as indices of the relationship of angiosperms. It was, therefore, deemed possible that such a study of the development of *Garrya* (combined with a thorough re-examination of its morphological features by modern methods) might throw important light on the phylogenetic relationship of this genus. It was with this hope that the research reported below was undertaken.

The work was done during the years 1924-5 and 1925-6 in the Botanical Laboratory of The Johns Hopkins University. Three months of this time (i.e. from December, 1925, to March, 1926) were spent in Berkeley, California, where the plants were studied in their natural environment in the Redwood and San Leandro Canyons. The work was undertaken at the suggestion of Professor Duncan S. Johnson and carried out under his supervision.

II. MATERIAL AND METHODS.

The material used was collected in California. Some of the fully matured fruits were gathered by Professor D. S. Johnson near Carmel in September, 1915, while he was a guest investigator at the Coastal Laboratory of the Carnegie Institution of Washington. Several younger stages were secured for him by Mrs. Wanda P. Vestal near Palo Alto in 1920, 1921, and 1924. Material was collected by the writer during the months of December, 1925, January and February, 1926, but much of this at present remains unexamined. The major part of this work, however, is based on material obtained for the author by Professor H. E. McMinn, of Mills College, Oakland, California, from the 'wild', about ten miles north of the college.

This material consisted of a series of specimens of *G. elliptica* collected, at weekly or bi-weekly intervals, from the latter part of October, 1924, until June, 1925. A portion of each collection was immediately forwarded by air mail; the remainder was at once fixed by Professor McMinn in formalin-alcohol and forwarded by parcel post. The usual time required for the air mail journey of the living flower and fruit clusters approximated two and one-half days. The specimens were carefully wrapped in wet cotton and paraffined paper, and generally arrived in excellent condition—fresh and turgid. The controls proved this method to

be entirely satisfactory for morphological studies, excepting in two instances, when the mail planes were delayed by storms. In these two instances the control material fixed by Professor McMinn proved necessary. In cases of delay, better results were secured by cutting the stems of the fresh catkins and allowing the material to stand in a cool place with stems immersed in water for twenty-four hours before fixing.

Portions of the material were fixed in formalin-alcohol (6 cc. commercial formalin to 100 c.c. of 50 per cent. alcohol). Other portions were fixed in weak chromo-acetic. The former fluid hardened the material slightly but did not discolour it; the latter rendered the ovules smoky black in colour unless these were first dissected from the ovary or a hole were made through the ovary wall to the surface of the ovule; with which treatment, however, the ovules remained uncoloured. The writer, at present, cannot account for the discoloration of the ovules under the conditions mentioned above. The dehydrated material was embedded in paraffin and sectioned in the usual way. The stains used were Delafield's haematoxylin and Heidenhain's iron-haematoxylin, both of which proved satisfactory. The figures are reproduced from drawings made with the camera lucida and from photographs. The degree of magnification of the figure as printed is noted in each case.

The flowers of *Garrrya* offer many obstacles to micro-technical preparation:

(1) The catkins, even in the earliest stages collected, are so strongly protected by the innumerable stone-cells (of the bracts) that it is impossible to cut microtome sections of them.

(2) From many epidermal cells of both bracts and ovaries arise long bent hairs, so thick-walled as to be practically solid excepting at their bases, where they are bulbous and comparatively thin-walled. Not only do these hairs resist the knife, but they are so densely packed that almost no space exists in or between them for the penetration of the paraffin matrix. Consequently the specimens drop from the paraffin of the sections.

(3) The ovaries, when young, are so tightly pressed by each other, the rachis and the bract, that they are variously flattened, and it is impossible in cutting to so orientate the material as to insure getting sections in the exact plane desired. The ovary as it grows experiences also a distinct torsion, which increases this difficulty.

(4) The flowers in a single catkin, or in different catkins collected at the same time from the same locality, show little, if any, variation in the stage of development. Hence a separate collection is necessary for each stage; and the plant, as has been said, is difficult to secure because of its habitat.

(5) In the mature seed the endosperm is exceedingly hard and resistant, which makes cutting microtome sections of the entire fruit wellnigh impossible. These several difficulties were overcome to some extent (*a*) by

dissecting the flowers from the catkins (a difficult process in the very early stages); (*b*) by embedding these in paraffin, then scraping thoroughly to remove the hairs and re-embedding; and (*c*) by removing the ovules themselves from the ovary at as early a stage as possible. It was observed that ovules which are fixed in formalin-alcohol, or are isolated from the ovary before fixation in chrom-acetic, became almost transparent in xylol; consequently, in cleared older ovules, the vascular bundle, the micropylar canal, and the outline of the endosperm are clearly visible with a hand lens. This removed the difficulty attending the orientation of the older ovules.

By these methods it was found possible to trace the development of the flowers and fruit from the first collection of material in October until complete maturity in the following September.

The ethyl and butyl alcohol, and other methods generally used for softening resistant material were thoroughly tried, and were found ineffectual in this case. Fixation in Carnoy's fluid was resorted to where it was necessary to secure sections promptly, but the results secured by the less satisfactory fixation thus obtained were always verified by comparison with specimens fixed in the chrom-acetic fluid. For older embryos, even after cutting away as much of the endosperm as possible, at least a half-hour in Carnoy's fluid was needed to secure thorough fixation.

In collecting material for a cytological study of *G. elliptica* it was noted that the method of transferring specimens directly from the tree to the fixing fluid is excellent if the specimens are cut when soil moisture is abundant, but the method is valueless if the specimens are picked during a drought. In this latter case the older embryo sac is flaccid and retracted from the integument which encloses it. Better results are secured by immersing the stems of the catkins or of the catkin-bearing twigs in water and standing in a cool place for twenty-four hours or longer before fixing the flowers.

The author wishes to express here her sincere appreciation of the interest and assistance, during the progress of this work, of Professor Duncan S. Johnson. She also wishes to thank Professor H. M. Hall, of the University of California, for suggesting to her Professor H. E. McMinn, of Mills College, California, through whose intelligent and faithful aid she was able to secure the many series of specimens used in this study and to gain valuable information concerning the habits of the growing plant. Professor Setchell of the same University is here thanked for putting the facilities of the University of California at the writer's disposal during her stay in Berkeley. Miss Alice Eastwood, of the California Academy of Sciences, rendered the writer valuable assistance regarding the taxonomy and geographical distribution of *Garrya*. She acknowledges also the courtesy of Professor LeRoy W. Abrams, of the Stanford University, who arranged for the collection of

material by Mrs. Vestal, and that of Mr. C. E. Miller, of the Johns Hopkins Medical School, for generous aid regarding technical difficulties encountered in the work.

III. OBSERVATIONS AND RESULTS.

General.

G. elliptica, as is well known, is native to California and Oregon only, but the staminate plant is widely cultivated in Europe because of the beauty of its long pendent catkins, which drape the tree at blooming time with yellow-green fringe (Pl. XXXVI, Fig. 1). The oldest plants seen by the writer were distinctly tree-like; occasionally attaining a height of twenty feet and a trunk diameter of eight inches (Pl. XXXVI, Fig. 2). The younger plants, however, were usually more shrub-like, and arose from the ground in dense clumps of tall slender trunks. The very young specimens had the appearance of stiff shrubs. In occasional isolated young plants the tree form still persists. The shrub-like form of the plants is possibly due to the depredations of wood-rats, which persistently use as food the top branches of both young and old plants. *G. elliptica* is one of the forty or more woody plants that make up the dense, almost impenetrable, chapparral covering large portions of many hills near the coast. The wood is 'non-zonate' (a fact also recorded by Lindley, Solereder, and others). The number of staminate and pistillate trees is, contrary to tradition, approximately equal, at least in the region studied. A record made of the flower-bearing trees of *G. elliptica* growing in this region showed sixty-one pistillate and seventy-two staminate trees. This count is, however, only approximate, since it was often not easy to determine whether a clump of small trunks represented one or several plants.

The leaves are oval with short tips; they are opposite, exstipulate, thick, and evergreen, shining and smooth above, but coated with a dense mat of hairs beneath. The edge is entire but curled in places, which makes the margin seem undulate.

The flowers are dioecious. Each staminate flower has four yellow stamens alternating with the four yellow perianth parts which are strap-shaped and clothed at the tips with stiff hairs (Text-figs. 4, 5). The pistillate flower consists of a single, usually bicarpellate, densely tomentose ovary surrounded by two or, in about 3 per cent. of the cases, three styles. The flower is naked excepting for the one or two circles of rudimentary tooth-like projections surrounding the base of the styles (Text-figs. 6, 8, and 9).

The nature of these organs will be discussed later.

Both staminate and pistillate flowers occur in groups of three. Each group is subtended by one of the decussate, connate bracts which form

a permanent portion of the catkin and fruit cluster (Text-figs. 6, 7, and Pl. XXXVI, Figs. 15, 17). The entire inflorescence has the form of a catkin, but with cyclic instead of spirally-arranged bracts (Pl. XXXVI, Figs. 3-18). The flowers are doubtless anemophilous. McMinn, who has collected large quantities of pollen for hay fever studies, states that he has never seen insects visit the flowers, and that the pollen is light and the grains small. Moreover, the pistillate flowers are green, inconspicuous, and lack both perfume and nectar. The writer noted further that in the months of December and January, during which the bulk of the pollen is shed, insects are rarely, if ever, to be seen in the regions inhabited by *G. elliptica*. It was noted also that the styles so twist that the stigmas are turned upward. The flowers on the back, unexposed side of the catkins elongate the styles, which curve forward to an exposed position with the stigmas turned upward, and hence advantageously placed to receive pollen grains brought by air currents. These facts indicate that the method of the pollination of *Garrya* is anemophilous. The pollen is exceedingly light, being carried for long distances in scarcely perceptible air currents. It is abundant and short-lived. Only a small percentage of the grains collected were viable after three days in a desiccator. While all the staminate flowers on a given tree open at approximately the same time, those of a second tree, even a contiguous one, may blossom several weeks earlier or later.

Besides this inherent difference in blossoming time, two other variations may be seen: occasional staminate trees produce flowers of a distinctly yellower colour than the others, and rarely a pistillate tree is found which produces catkins which are noticeably longer and more slender than those of the average pistillate tree.

The seeds are disseminated by animals; perhaps in part by birds, certainly by wood-rats, as the seeds have frequently been seen, by McMinn and others, in the nests of these creatures.

The season of 1924 and 1925 proved an exceptionally favourable one for *Garrya* in the region of the Oakland Hills. The development was several weeks in advance of the average, the flowers and fruits produced in extraordinary abundance, and the pollen-shedding period unusually prolonged.

The Catkins.

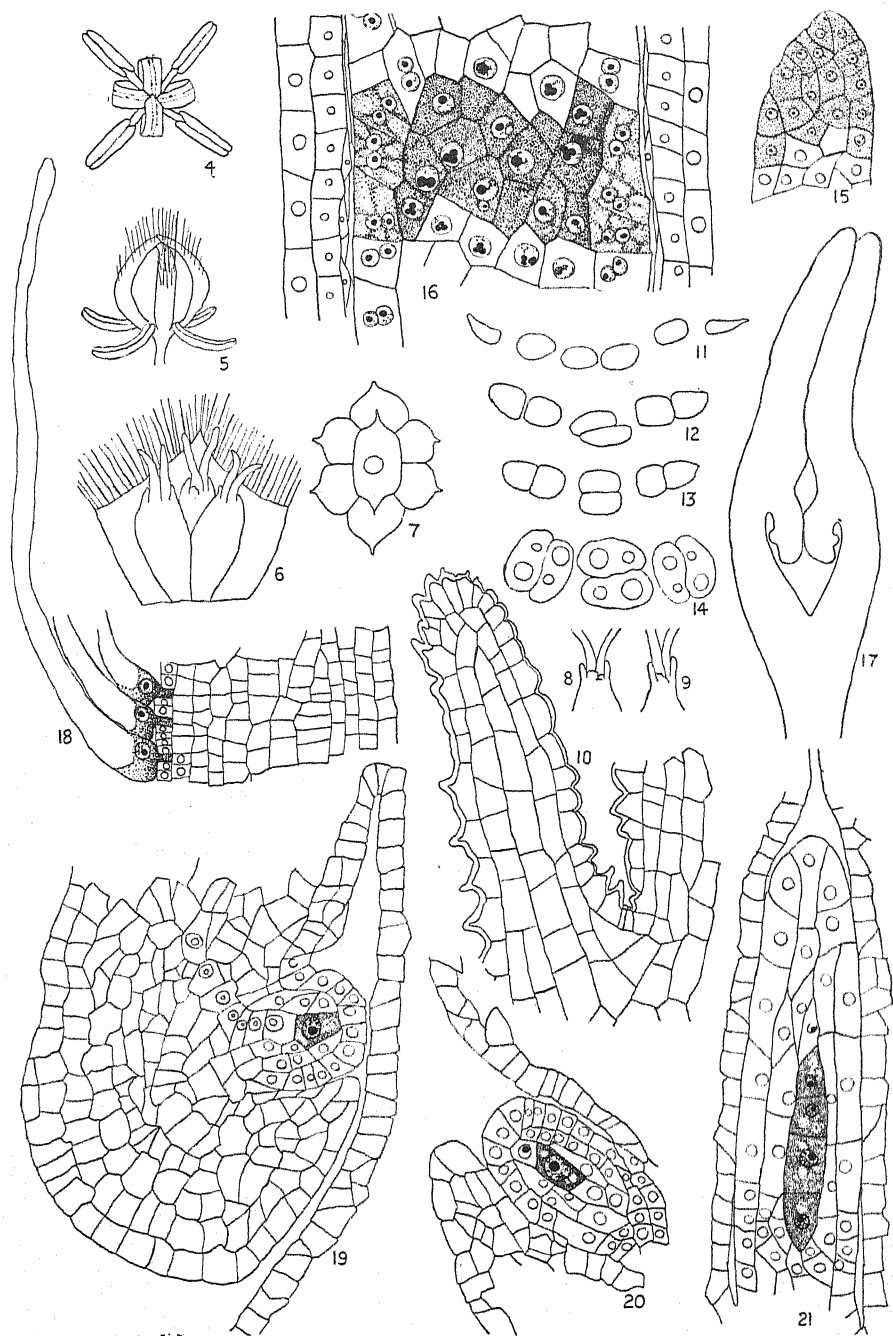
In October both staminate and pistillate catkins are green, rigid, and of equal length (Pl. XXXVI, Figs. 4, 5), the longest of each scarcely reaching $1\frac{1}{2}$ cm. The bracts are closely appressed and give no external evidence of the underlying flowers. The staminate catkins are terete (cylindrical) with slightly convex bracts. They are less rigid and dense, and are generally light enough to float for a time on the surface of the chromo-acetic fluid.

The staminate catkins developed rapidly in early autumn, and in mid-November flower buds were seen emerging, three from behind each bract, and supported on rapidly elongating pedicels (Pl. XXXVI, Fig. 6). The internodes of the catkin also elongate and separate the flower clusters sufficiently to permit of the complete development of each flower. By the first of December an occasional catkin had attained a length of 17 cm., and was already shedding pollen. The figures published by Lindley, Decaisne, Baillon, Engler and Prantl, and others, give an excellent idea of the staminate catkins at this stage.

The pistillate catkins are dense, four-cornered, with flat lateral faces, and the median lines of the bracts form the corners of the catkins. The tips of the bracts at the base of the catkin are often elongated and curve outward and down. The size of these tips gradually decreases towards the apex of the catkin (Pl. XXXVI, Figs. 5, 7, and 10).

The pistillate catkins develop slowly. Their approximate rate of growth is shown in the table given later (p. 783). They become less definitely angled, and early in December the three pairs of styles (one pair for each of the three flowers subtended by the bract) are so elongated that they protrude from behind the bract (Pl. XXXVI, Fig. 8). Occasionally one, sometimes even two, of these pairs fail to push from behind the bract, and the flowers represented by these develop no further. This happens most frequently in the upper third of the catkin, with the result that, in this region, bracts are frequently found which subtend only one or two fruits, while in the lower part of the catkin all the flowers develop as a rule.

The styles are at this time straight, pale green, translucent, tapering cylinders which elongate rapidly, are somewhat recurved, and by mid-December have developed reddish, glistening stigmatic surfaces for the reception of pollen. Though the typical number of styles is two, the flowers near the middle of the catkin frequently possess three well-developed styles. The catkin enlarges slowly and gradually loses all trace of the four-sided prismatic form. Early in February the tips of the ovaries show above the margin of the bract (Pl. XXXVI, Fig. 12). By the end of the month the pedicels of the pistillate flowers are somewhat elongated and the ovaries are slightly swollen and are exposed for about one-half of their length (Pl. XXXVI, Figs. 13, 14). The catkins, even of isolated pistillate plants, develop as far as this. They remain green with styles recurved and stigmas apparently receptive for several months. At the beginning of the following growing season these catkins may be seen now dried up, hanging by hundreds on the trees. Two weeks later the ovaries of pollinated flowers show an almost doubled size, so that the margins of the subtending bracts are forced outward and downward into a nearly horizontal position (Pl. XXXVI, Figs. 15, 16, and 17). The rapid development continues during the following month, at the end of which the ovaries stand as crowded tomentose globes each on a short



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TEXT-FIGS. 4-21. 4. Staminate flower (Dec. 12) as seen from directly above. Perianth parts still united at tips. $\times 1\frac{1}{2}$. 5. Lateral view of same flower. $\times 2$. 6. Adaxial face of bract of pistillate catkin with its three female flowers with perianth parts of each. $\times 5$. 7. A pair of connate bracts seen from below showing positions of the three mature ovaries subtended by each bract. 8, 9. Abaxial faces of tips of two ovaries (Dec. 26, 1924) showing two sorts of perianth parts. $\times 5$. 10. Longitudinal section of part of style of a pistillate flower (at right) and of a single perianth part

pedicel hidden by the bract. They are still green at this time and possess two large conspicuous veins which traverse the ovary wall from the pedicel to the styles, and either one or two fainter ones parallel to these. The larger veins always appear on the exposed surface of the globe and form with each other angles of from forty-five to one hundred degrees. The larger sepals stand on these veins as a rule (Pl. XXXVI, Figs. 17, 18).

The comparative rates of elongation for the staminate and pistillate catkins are shown in the accompanying table. The development of the staminate catkin ceases, of course, when the pollen is shed. The longest, central catkins of a cluster (rather than an average of all) were recorded, since the laterally placed catkins often fail to develop.

*Comparative Rates of Growth of Staminate and Pistillate Catkins of
Garrya elliptica.*

Length in Centimetres.	Oct. 29.	Nov. 14.	Nov. 28.	Dec. 12.	Jan. 16.	Feb. 14.	Mar. 12.	Apr. 14.	May 12.	June 16.
	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.
♂ Catkins	1½	5	12	18						
♀ Catkins	1¼	2½	4½	5	6	8½	9	10½	10½	10½

Flowers.

The staminate and pistillate flowers are easily distinguished, even in October. The former are larger, obovoid, and with all parts clearly developed. The latter are embedded in a dense tuft of short hairs and distinguishable only by the small, white, projecting styles which at this time exceed the ovary in length.

The growth of the staminate flowers is rapid, keeping pace with that of the catkin as a whole. The flower buds emerge; the four yellow, strap-shaped perianth parts still cohere at their tips but separate at the

(at left) showing its internal structure and its position with relation to style (Dec. 12). × 40. 11, 12, 13. Transverse section through styles of cluster of three pistillate flowers at three successively lower levels, showing torsion of the central of the three flowers (Jan. 16). × 12. 14. Transverse section through ovaries of same flower cluster showing position of ovules and embryo sac and raphe in each. × 12. 15. Longitudinal section of cone of sporogenous tissue in young anther (Oct. 29). × 150. 16. Part of a similar section of anther (Nov. 12) showing three layers of parietal cells, also differentiation of sporogenous tissue into binucleate tapetal cells and uninucleate definitive sporogenous cells. × 200. 17. Longitudinal section of young pistillate flower (Oct. 29, 1924) showing beginning of formation of nucellus. 18. Part of longitudinal section of wall of young embryo (Dec. 12) showing four outer and four inner layers of cells and zone of irregular cells between that become sclerenchymatous as the fruit ripens; and showing also the structure of hairs and their origin in epidermal layer of wall. × 150. 19. Sagittal section through ovule (Nov. 14) showing primary archesporium, nucellus, and integument just initiated. × 200. 20. Sagittal section through ovule (Nov. 14) showing definite archesporial cell and tapetal cell. × 200. 21. Sagittal section through ovule (Dec. 12) showing row of four potential megaspores, nucellus, and inner cell layer of integument. × 200.

middle, thus revealing through the resulting apertures the four yellow stamens. Next, the short filaments curve outward and downward, thus bringing the anthers into an oblique position, pointing outward and downward till the tips lie slightly below the level of their insertion (Text-figs. 4, 5). Then the perianth divisions separate completely and turn outward to lie in an almost horizontal plane. The anther chambers now open by longitudinal fissures and the pollen is shed. It is an interesting fact, the significance of which is not yet known, that ripe staminate catkins if placed in 70 per cent. alcohol slowly yield a pigment which in alcoholic solution is dark blue by transmitted daylight and which shows red also by transmitted artificial light. The nature of this pigment was not determined beyond the facts that it is not chlorophyll, is not soluble in water, oils and lipoids, is unchanged by acid and alkali, and that the absorption spectrum shows that it transmits the blue and green as well as the red, but cuts out the yellow almost completely and exhibits dichroism very prettily. The colour persists in the alcohol for some months, then, perhaps because of oxidation, the dichroism gradually increases and becomes clearly visible by daylight. Both red and blue finally disappear and the pigment turns black. This pigment is produced by the vegetative organs of the plant also, but in such small quantities as to be quickly masked by the chlorophyll.

The growth of the pistillate flower is slow. The ovary is about the length of the styles at the time when these emerge from behind the bract (Text-fig. 6). Each flower, when dissected from the bract, usually shows several small hard teeth projecting from near the tip of the ovary, beside the styles. These teeth vary in size, and in some cases are practically hidden in the mass of hairs which covers the ovary wall, and which forms at this time fully one-half the bulk of the ovary. The development of these teeth accompanies that of the ovary, and they become easily visible in most cases as hard green opaque projections extending about 2 mm. above their insertion on the ovary. They vary in number, but there are generally at least two or three teeth on each ovary (Text-figs. 6, 8, and 9). Microtome sections showed that these teeth stand close to the base of the styles (Text-fig. 10). This figure represents a longitudinal section through the 'foliole' and shows its internal structure. It consists of five layers of cells covered by a typical epidermis, certain cells of which are elongated into reduced but entirely typical hairs. Small fibrovascular bundles run into the bases of these teeth, as can be seen by longitudinal sections of the ovary wall.

An examination of the flower at a stage just before the tip of the ovary emerges from behind the bract revealed a second set of projections alternating with those described above, and standing between them and the styles. These were more delicate, translucent, cream-coloured, with but a slight tinge of green. In three cases the colour was distinctly rose with no trace of

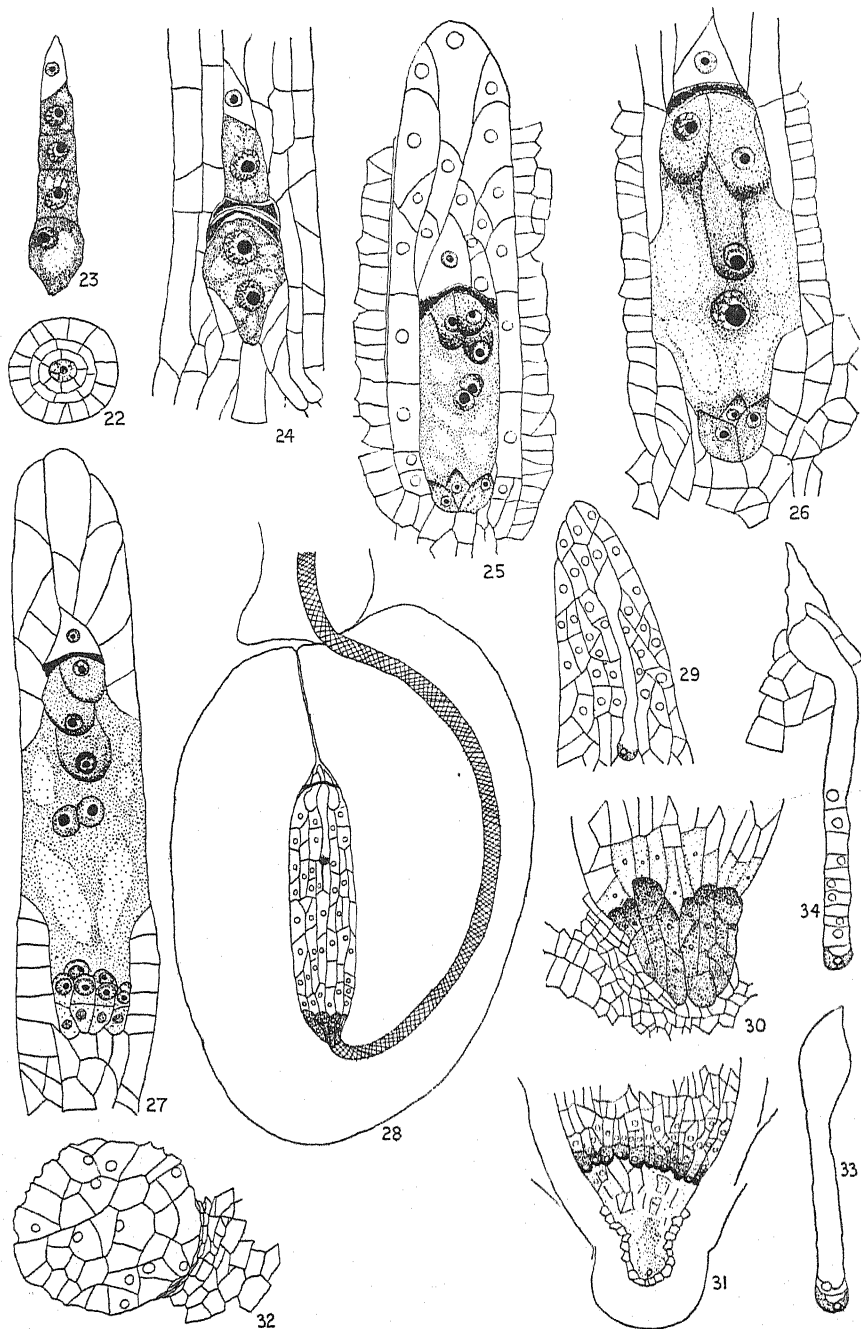
green. These teeth of the inner row dried up after a few weeks. The outer ones persisted unchanged until the ripening of the fruit.

Ovules.

The large majority of ovaries possess two styles and contain two ovules; however, in some of these only one ovule is formed. (In the ovaries with three styles three ovules are invariably found.) The ovules are elongated, and each is pendent from a long funiculus (Text-fig. 28). This stalk is swollen at a little distance from the placenta and forms an 'obturator', which overhangs, without touching, the micropylar end of the ovule. The cells composing the obturator are large, spherical, and arranged in a large grape-like cluster. The general structure of the older ovule and arrangement of the ovules within the ovary was easily made visible by clearing individual flower clusters in xylol after the ovaries had been opened as described above. In each nearly transparent ovule the projecting funicle sweeps around one side of the ovule. Its fibrovascular bundle is rather deeply embedded and may be seen as a distinct brown strand running parallel to the surface of the funicle. At the base of the ovule the bundle swings inward and upward to the chalaza—i. e. to the base of the nucellus.

By February or early in March the embryo sac is a long straight spindle definitely outlined in the cleared ovule by distinct brown lines, indicating the inner surfaces of the integument. These lines meet at the top of the embryo sac and continue on beyond to the surface of the ovule to outline the walls about the micropyle (Text-fig. 28). The arrangement of the ovules in the ovary varies with the location of the containing ovary, but the relation of the ovules to each other is constant in the ovaries which contain but two. The ovaries, as has been said, occur three behind each bract. In the centre one the paired ovule lies with its long axis practically parallel to the axis of the rachis and its flat median face tangent to its surface (Text-fig. 14). As the ovules are identical, the positions of the embryo sac and raphe are reversed in the two, so that a cross-section of the ovary shows the vascular bundle of the one opposite the embryo sac of the other. In the ovaries on each side of the central one the ovules are arranged at approximately right angles to those in the first, that is, a plane passing between their contiguous faces would include the axis of the rachis. The arrangement of the ovules in these two side pairs is reversed—that is, their positions are the same with relation to the central pair. The ovules nearest the central pair turn their raphes towards the rachis and their embryo sacs towards the bract, the outer ovule of each pair being, as was said, in a position the reverse of this (Text-fig. 14).

When one or three ovules develop, the position of these seem, as far as has been determined, to follow no fixed rule. The solitary ovule is oblong



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TEXT-FIGS. 22-34. 22. Transverse section of above showing megaspore, two layers of nucellus, and inner cell layer of integument. $\times 150$. 23. The chalazal megaspore (Dec. 20) enlarging to form the functional one. $\times 200$. 24. Degeneration of two intermediate potential megaspores (Dec. 20) and division of nucleus of functional megaspore. $\times 200$. 25. Longitudinal section through nucellus and part of integument of ovule (Dec. 20) showing eight-celled embryo sac. Inner layer of lateral nucellus cells absorbed. Composite drawing from two successive sections.

and curved over its entire surface. When three develop, their tips (also their bases) lie at three different distances from the styles.

The Staminate Flower.

The Microsporangium.

The early development of the stamens was not studied, for the youngest material collected, namely, that which was secured in late October, shows the staminate flowers already completely formed. The perianth parts are well developed. These enclose the stamens, which are now fully differentiated into filaments and anthers. Each anther includes four already mature microsporangia. A longitudinal section through a stamen shows in each microsporangium the primary parietal layer divided periclinally to form three concentric parietal layers. The inner layer is greatly reduced and is occasionally lacking. The cells of these layers are elongated parallel to the surface and are flat and uninucleate. The primary sporogenous tissue enclosed by the parietal layers is sharply defined (Text-fig. 16). It is an elongated cone of cells extending practically the length of the sporangium. In the axis of the cone the cells are slightly elongated and arranged in imperfect longitudinal rows. The cells nearer the surface of the cone are angled and irregular. The primary sporogenous cells are at this time all approximately equal in size and contents (Text-fig. 15). However, a week later the cells formed from the periphery of the primary sporogenous tissue are invariably binucleate and highly vacuolated. These are the tapetal cells, and they contrast sharply with the inner, the definitive sporogenous cells, which are uninucleate and have dense, non-vacuolated, deeply-staining cytoplasm (Text-fig. 16). The entire tapetum here consists, therefore, of sterile cells cut off from the periphery of the sporogenous mass. Both tapetal and definitive archesporial cells divide several times. The new walls are irregularly placed so that the resulting tapetal cells and spore mother-cells form a continuous mass of irregular, sharply angled and closely packed cells; and the two sorts of cells are distinguishable only by the difference in their positions and in their protoplasts (Text-fig. 16).

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- × 200. 26. Longitudinal section through embryo sac (Jan. 16) showing polar nuclei fused, and middle and lateral portion of nucellar tissue completely absorbed. × 200. Composite drawing from two successive sections. 27. Longitudinal section through embryo sac (Feb. 5) showing endosperm nucleus and antipodal cells divided. × 200. Composite drawing from three successive sections. 28. Optical sagittal section of ovule (Feb. 5) cleared in xylol, showing raphe with its well-developed vascular bundle, obturator, integument, micropyle, embryo sac containing remains of degenerated megaspores, egg apparatus, antipodals, and endosperm cells, which has almost completely replaced the nucellus. × 10. 29. Sagittal section through tip of embryo sac (Feb. 14) (endosperm mass of above) showing pro-embryo with long suspensor embedded in endosperm cells. × 75. 30. Antipodal portion of same section showing endosperm cells above, and groups of cells arising by the division of the antipodals below. 31. Chalazal end of embryo sac (Feb. 28) showing deterioration of antipodals and corresponding enrichment of the adjacent endosperm cells. × 50. 32. Transverse section of endosperm mass (Feb. 12) showing its growth to crush back the inner cells of the integument. × 75. 33. First division of fertilized egg or pro-embryo (March 12). × 75. 34. Three-celled embryo and eight-celled suspensor (March 24). × 75.

The massula-formation is not conspicuous. The mother-cells do not at first become separated but remain closely packed in the sporangium. The actual mitosis of the pollen mother-cell was not evident in the material examined; however, numerous pollen mother-cells were seen in which four nuclei were grouped in the centre without dividing walls between. This would indicate that the division of the pollen mother-cells is by the 'simultaneous' method, which, according to Coulter and Chamberlain, predominate among Dicotyledons. Walls are laid in, which separate the four nuclei of the pollen mother-cell, and a typical tetrad is formed. The pollen mother-cells are now rounded and separated from each other. The space around and between them is occupied by the continuous mass of protoplasm which is produced by the disintegration of the tapetal cells and which is known as the periplasmodium.

The main portion of the periplasmodium disappears soon after its formation, but the portion filling the slender elongated tip of the microsporangium persists for some time.

The walls of the microspores are thin at first. The spores round up and separate and almost immediately differentiate into exine and intine. The exine is thin but finely sculptured with numerous pits. It is penetrated by four sprouting pores tetrahedrally arranged. The microspore contains at this time a single large nucleus and has highly vacuolated cytoplasm. Later the nucleus divides and forms two similar daughter nuclei. Very soon the usual differentiation of these nuclei occurs. The tube nucleus enlarges and shows a large nucleolus but rather scant chromatin. The generative nucleus does not divide inside the pollen grain nor had it within the short, young pollen-tubes which were observed. The division probably takes place at a later stage when the pollen-tube has attained a considerable length.

By the time the tetrahedral division is completed the outermost parietal layer has thickened slightly. The parietal layers separating two adjacent sporangia of each half anther are flattened, then ruptured, so that the two cavities are merged. Later the pollen sacs dehisce by longitudinal fissures extending the full length of the anther. The lips of these fissures curl outward and the ripe pollen is exposed and then scattered.

The Pistillate Flower.

The Megasporangium and Ovule.

The writer was unable to study the early development of the pistillate flower. A longitudinal section of a flower collected in October shows the ovarian cavity completely enclosed by the carpels and even the styles well developed (Text-fig. 17). The ovary wall consists of four outer layers of cubical cells, four inner layers of similar cells, and a zone between composed

of cells irregularly placed (Text-fig. 18). These cells later become sclerenchymatous and form the middle layer of the wall of the fruit (Text-fig. 48). During February the ovary wall becomes loosely fitting, the opposite inner faces meeting for a considerable distance beside and below the ovules. This illustrates an interesting adaptation to the sudden enlargement of the ovules which occurs later. The ovary wall is thicker below the styles and above the pedicel. Many of the epidermal cells of the ovary are elongated into long, stiff, bent hairs. The ovules are already elongated, each pendent from a lateral placenta. Sections of the youngest pistillate flowers collected showed the formation of the nucellus to have already begun (Text-fig. 17). The nucellus at this time takes the form of a low dome of cells projecting towards the ovary wall from the side of the ovule, at a region near its lower or free end. This dome elongates into a protuberance which is about 5 cells in width and 6 cells in length, inclusive of the single layer of epidermal cells which covers the whole a week later. The cells of the axial row are slightly differentiated by increased size and by the density of the protoplasm. The terminal cell of this axial row, the hypodermal cell, is almost immediately sharply distinguished as the primary archesporium, not only by its increased size and especially dense, deeply-staining protoplasm, but by the nucleus, which is now exceedingly large, and contains an unusually large nucleolus (Text-fig. 19).

Meanwhile an annular outgrowth has formed at the base of the nucellus. This outgrowth develops rapidly to form the single integument, which soon overtops and completely encloses, save for the micropylar canal, the peg-shaped nucellus (Text-figs. 21, 28). The growth of the integument is more rapid on the side away from the placenta, consequently the nucellus and micropyle are gradually turned upward in a line with the placenta (Text-figs. 21, 28, and 47). Meanwhile the primary archesporium divides by a transverse wall and produces the hypodermal tapetal cell and the definitive archesporium beneath it (Text-fig. 20). The tapetal cell divides no further but is depressed somewhat into the nucellus by the subsequent elongation and periclinal divisions of the adjacent nucellar cells. The definitive archesporial cell divides by a wall transverse to the ovule to form two daughter cells. Each cell divides again transversely; so that an axial row of four long potential megaspores is produced, which are separated by distinct cell walls. The primary tapetal cell remains as an integral part of the row and closely resembles the megaspores in position, form, and contents (Text-fig. 21). A longitudinal section of the ovule at this time shows a massive integument (of from 15 to 25 cells in thickness) inclosing a peg-shaped nucellus lying in a line with the long slender micropylar canal. In the centre of the nucellus, parallel to its long axis, lie the row of four potential megaspores and the tapetal cell. The nucellus itself consists of two layers of long fragile cells except at its tip and base, where the cells

are less fragile and more numerous (Text-fig. 21). A complete section also shows that certain cells of the funiculus have proliferated rapidly in the region slightly below the placenta, and have formed the 'obturator' which extends out laterally like a canopy above, but does not touch, the micropylar end of the ovule (Text-figs. 28, 47).

The four potential megaspores persist unchanged for a short time. Then the inner one, that nearest the chalaza, gradually enlarges, rounds up somewhat, and becomes the functional megaspore (Text-fig. 23), while the three micropylar potential megaspores degenerate and collapse (Text-fig. 24). They do not entirely disappear, however, but remain as a densely-stained cap covering the micropylar end of the functional megaspore, even till the embryo sac is nearly mature. The nucleus of this megaspore, by three successive divisions, produces an eight-celled embryo sac (Text-fig. 25). The eight-celled embryo sac is large, much elongated, and typically bipolar. The three antipodals with their dense contents are closely packed in the narrowed chalazal end of the sac. Their tips extend outward into the sac as angular, sharply-defined points. The three cells of the egg apparatus are all elongated; the egg cell itself is especially so, for it extends almost to the centre of the sac. The nucleus lies in the tip, which is slightly swollen and contains the bulk of the protoplasm. The polar nuclei fuse near the centre of the sac to form a large endosperm nucleus, sometimes 15 micra in diameter.

The embryo sac remains in this seven-celled stage for about five weeks. During this time it slowly enlarges at the expense of the lateral nucellar cells beside it, so that at the end of this period there remains no trace of this portion of the nucellus, but the longitudinal walls of the enlarged embryo sac lie closely pressed against the inner surface of the integument itself (Text-fig. 26). The nucellar cells at the two ends of the embryo sac remain turgescient and healthy. Meanwhile the cytoplasm near the two ends and immediate centre of the embryo sac becomes so dense and stains so deeply that it almost conceals the nuclei located in these regions.

Many of the embryo sacs do not advance beyond this stage, so that it is not unusual to see two ovules side by side, the size of one many times that of the other. The small ovule does not degenerate but remains externally perfect until the other develops to a ripe seed. The small ovule always undergoes a still further external development, which will be described later.

Whether the failure of certain ovules to mature is due to lack of fertilization or to a cause similar to that unknown cause which checked the development of numerous young flowers is not determined. The same phenomenon has been repeatedly noted by many authors. Bower (1908) discusses this for seed plants in general and Harris (1909-10) attempts to account for it as it occurs in *Cercis canadensis*. According to Johnson

(1910, pp. 717, 724) the reduction is marked both in the stamens and ovaries of *Piper betel*. The latter may possess completely closed cavities without a trace of an ovule. In *G. elliptica* the pistillate flowers seem regularly to produce normal ovaries, even in the young flowers, which cease growing several weeks before the pollen is mature.

Fertilization.

Flowers of isolated pistillate trees were thickly dusted with fresh pollen, collected from the nearest staminate trees. The pollen was secured by catching groups of hanging catkins in a small aluminium funnel with the stem covered by a glass vial. The catkins were shaken gently and a layer of pollen was deposited on the inner surface of the funnel. The funnel was then tapped gently till the pollen slid down into the vial. The lightness of the pollen and its short period of viability necessitated this method. Pistillate flowers so pollinated were collected at approximately two-day intervals and the growth of the pollen-tube was studied in microscopic sections of the stigmas (as well as in artificial media).

The growth of the pollen-tube is slow. Stigmas collected two days after pollination show the pollen-tube emerging from a sprouting pore of the pollen grain as a slender tube densely filled with protoplasm. Two days later the tubes are seen penetrating the outer cell layers of the stigma. Ten days after pollination the tubes extend, in some cases to the base of the style. Seventeen days after pollination the pollen-tube extends the entire length of the long micropylar canal, and its end is seen poised directly above the apex of the nucellus. The slender tube is still densely filled with protoplasm, and the nucleus, though small, is clear and distinct. At this time the styles upon which the pollen-tubes have fed shrivelled and dried. Longitudinal sections through them show them to be dead and unstained almost to their bases. The line of demarcation across the style between the dead and living portions of it is sharp and definite, and the dead portion of the style is easily separated from the living part by a slight touch.

The actual union of the male nucleus with that of the egg has not as yet been observed. It is possible that it is this union which ends the long resting period of the embryo sac or possibly the rains which fell in late January after a long period of dry weather. However, at the end of the first week in February, a series of important changes occur in the sac. These changes took place with such rapidity and started with such unexpected suddenness that it was impossible to arrange for the close succession of collections necessary to afford a complete record. However, enough steps were seen to allow the practically certain determination of the real sequence of stages. First the endosperm nucleus divides repeatedly

(Text-figs. 27, 28, 30, and 31). The two, four, eight, and sixteen nucleated stages of the endosperm were seen. In these stages the nuclei show no preliminary migration to the wall of the embryo sac, neither is the sac divided into two parts by a wall laid down after the first nuclear division. The nuclei simply migrate to all parts of the embryo sac, and upon reaching their destination continue their divisions with the formation of cell walls, and the cells are almost invariably uninucleate. The nuclei at first appear to be more numerous in the region of the egg apparatus, and the separating walls are laid down there more promptly. The cell wall formation lags somewhat in the region near the antipodal end of the sac, and occasionally small areas of protoplasm are seen there which contain several well-separated nuclei with no trace of cell walls between.

After the first 'rush' of divisions, the endosperm cells become uniformly uninucleate and the endosperm continues to enlarge by a steady, orderly division of its cells. The egg apparatus at this time is still unchanged. The synergids are turgescient and filled with dense protoplasm. The long tubular proembryo extends into the sac and is entirely embedded in the mass of endosperm (Text-figs. 28, 29). The endosperm cells surrounding the egg apparatus are large, numerous, and filled with dense protoplasm. The synergids closely resemble the enveloping endosperm cells at this time and later are not to be distinguished from them at all. The endosperm cells in the other portions of the embryo sac are now small with distinct walls and deeply staining nuclei, but with a cytoplasm so watery that it is barely perceptible with an ordinary stain. At about the time of the first division of the endosperm nucleus each of the antipodals enlarges and divides longitudinally. The six resulting cells elongate and each divides transversely (Text-fig. 27). The six of these twelve cells that project into the sac have large nuclei and thin walls. These cells continue dividing rapidly till they form a cell mass filling nearly one-fifth the cavity of the sac (Text-fig. 30). These cells at first have dense and dark-staining contents which contrast sharply with the highly vacuolated endosperm cells adjacent to them. Two weeks later, however, the antipodal cells are poor in contents and highly vacuolated, while the endosperm cells immediately adjoining them have dense and dark-staining contents (Text-fig. 31). There seems little doubt that these endosperm cells are being nourished at the expense of the antipodals which lie between them and the chalaza. For four weeks the innumerable endosperm cells continue to multiply and the entire mass increases rapidly, crushing back the inner cell of the integument (Text-fig. 32, 35), and pushing before it in its enlargement a broad band composed of the collapsed empty walls of the crushed integument cells. This band stains densely and darkly, and outlines clearly the embryo sac, not only in stained sections, but even in the entire ovule when it is cleared in xylol. The cells derived from the antipodals are now practically colourless with

almost invisible walls. It is possible that the contents of the cells in the base of this cup-shaped mass are now fused, for large bodies of protoplasm are frequently seen in this region, which may represent the accumulated degenerating contents of the cells near the chalaza. However, the entire mass is still strongly turgescient and offers a resistance almost equal to that of the slowly encroaching endosperm cells (Text-fig. 31). From this time until complete maturity of the seed the endosperm continues to enlarge slowly, but never entirely displaces the antipodal cells. The cells of the endosperm become at the same time denser and darker, and are bright green when fresh. By March 28 the endosperm appears (in ovules cleared in xylol) as a dense brown spindle with a transparent mass at the chalazal end. This transparent mass indicates the position of the highly vacuolated cells derived from the antipodals.

The Embryo.

The fertilized egg-cell (or proembryo), which has persisted for five weeks as an elongated slightly vermiform tube (Text-figs. 28, 29), now divides by a transverse wall which cuts off the tip of the cell (Text-fig. 33). Two further divisions occur in a plane parallel to the first, so that the proembryo now consists of a long transparent suspensor bearing on its inner or antipodal end a young embryo composed of three short broad cells.

The lower, antipodal half of the suspensor now divides repeatedly. The new walls are approximately transverse and the filament is divided into from ten to fourteen cells (Text-fig. 34). Later those cells of the suspensor which lie nearest the embryo, also those which lie in the middle of the filament, divide longitudinally (Text-fig. 36). Still later, longitudinal divisions may occur in any of the cells of the antipodal half of the suspensor, so that the suspensor ultimately consists of two definite parts; first, the micropylar half, which is a long transparent tube with the end next the micropyle slightly swollen; and second, the antipodal half, which is a cellular filament. Each segment of this filament consists of one or two cells or of a plate of four cells. The suspensor remains in approximately this condition until crushed by the maturing embryo (Text-fig. 37).

The three-celled embryo next divides longitudinally, forming three horizontal regions of two cells each. Almost immediately a second longitudinal division takes place at right angles to the first, so that each of these three transverse regions now consists of a tier of four cells (Text-figs. 36, 37). The cells of the apical tier are frequently angled and form points projecting into the endosperm. But these are later rounded up by the pressure of the adjacent endosperm cells. The cells of the apical tier next divide by periclinal walls which differentiate the dermatogen in this region; the cells of the tier adjacent to the suspensor undergo no corresponding division. The succeeding divisions of the embryo follow in the order

described for the *Capsella* type (Text-fig. 38), excepting that there is no hypophysis, the root-tip being covered by the four-celled plate formed by the division of the adjacent cell of the suspensor. All the cells of the embryo now divide rapidly without a correspondingly rapid increase in size, so that by April 14 a large spherical embryo is formed consisting of rectangular cells so small that the relatively large nuclei are at times somewhat flattened by the adjacent walls.

The spherical stage of the embryo persists in *G. elliptica* for an exceptionally long time. On April 28 some of the embryos showed sixteen cells along the transverse diameters, while the longitudinal diameter may have sixteen to eighteen cells with still no indication of the formation of cotyledons (Text-fig. 38).

The cells of the embryo continue to divide for several weeks longer, and the embryo increases considerably in size. A zone of modified endosperm cells, about ten cells in thickness, surrounds the young embryo. The cells of this zone are stained blue with Heidenhain's haematoxylin; the other endosperm cells are stained lavender. The contents of the modified cells is evidently liquid, and the walls are thin, while the other endosperm cells are now beginning to show starch grains among their contents. There is no sharp line of demarcation between the two regions of cells. It is possible that an enzyme is secreted by the embryo, which is effective for this distance. It is also possible that this is a specialized zone of the endosperm cells around the embryo, and functions as does the whole endosperm in *Saururus*, which is reported by Johnson to digest, absorb, and pass on food material from the surrounding storage regions. It is also suggestive of the single layer of 'jacket cells' surrounding the archegonium of *Cycas revoluta*, though the continuity of protoplasm between the inner and the surrounding cells is lacking here. However this may be, this zone persists until the embryo is mature, though it is then made up of quite different cells from those that constitute it at the beginning. Other cells are added to its outer margin as rapidly as those within are crushed and absorbed by the enlarging embryo. When the embryo is mature the cells immediately surrounding it are plump, stain darkly, and resemble the endosperm cells, except for the absence of starch grains within.

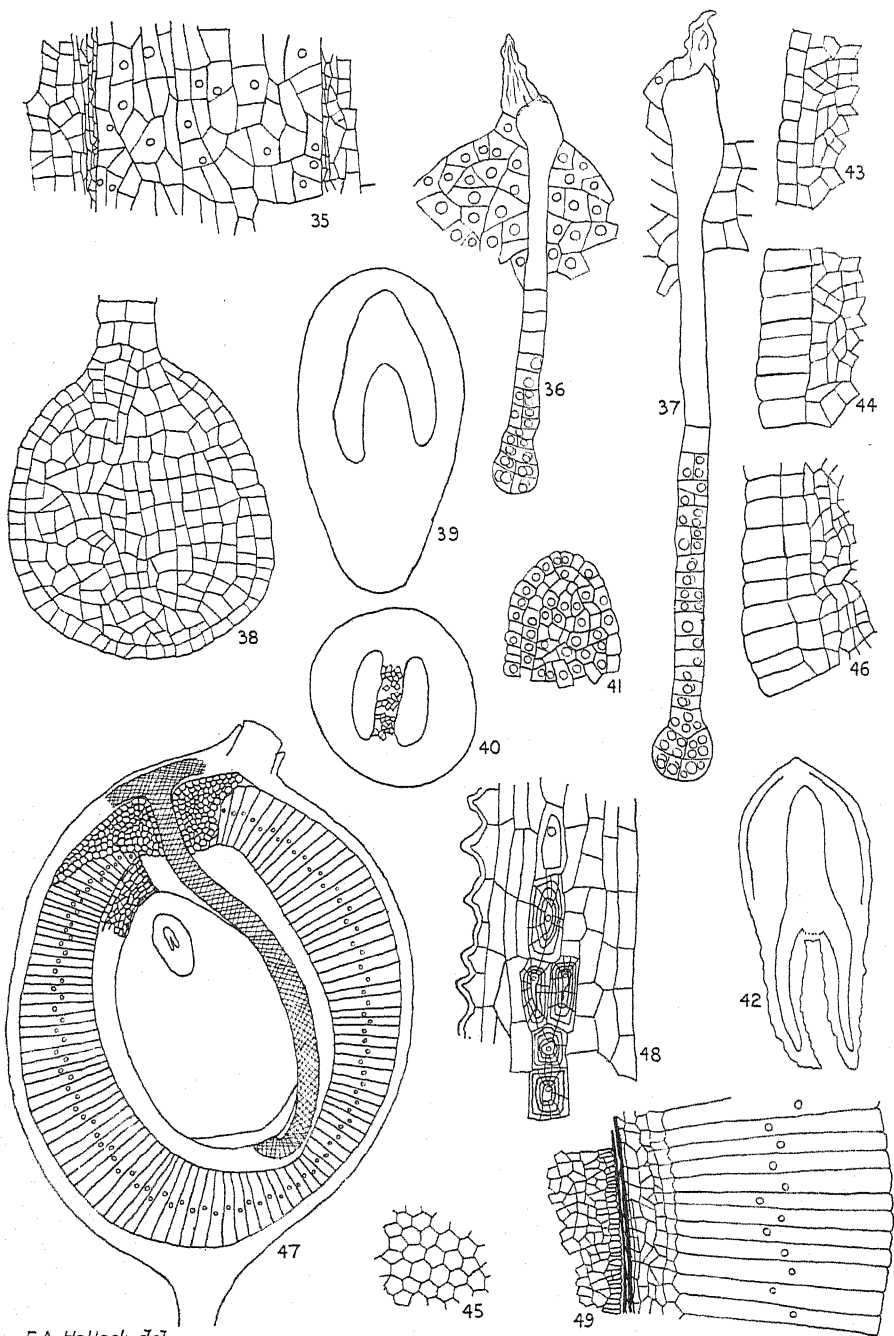
In the specimens collected towards the end of May the embryos show the first indication of the formation of the cotyledons. Cells proliferate in two regions of the antipodal end of the embryo in such a way that the free end of the embryo appears truncate. The projecting angles represent the two cotyledons. The cells forming these angles multiply steadily and elongate slightly. The cells between them multiply slowly. The cells at the base of the embryo multiply with a moderate degree of rapidity, so that at the end of about two weeks a longitudinal section of the embryo passing through the two cotyledons is shaped like a thick crescent which has blunt

tips and its outer margin towards the micropyle. From now until maturity there is a steady elongation of the embryo and a slower increase in width. By mid June the total length and breadth of the embryo are about equal, the cotyledons still have strongly divergent inner surfaces, and the space between them is filled with endosperm (Text-figs. 39, 40). The plerome is not yet differentiated, but there are definite indications of vascular elements (Text-figs. 41, 42).

In September the seed is entirely mature. The minute embryo is now approximately cylindrical in shape (Text-fig. 42). The ends, however, are bluntly pointed, and a slight constriction appears midway between them. The length of the cotyledons about equals that of the radicle. The long inner faces of the cotyledons meet (excepting at the base), and are separated only by a thin transparent layer of wasted endosperm residue. A longitudinal section of the embryo median to the cotyledons shows that the inner faces of the cotyledons diverge slightly at their bases, between which lies the stem-growing point. At this time this stem-apex is a broad, slightly dome-shaped region of undifferentiated tissue. Even in the embryo of a mature seed there is no indication whatever of a plumule or root-cap (Text-fig. 42).

The meristem regions of this mature embryo are well differentiated, but the mature tissues themselves are not yet specialized in them. The stele of the radicle is shaped like an inverted cone, with a rounded tip at the root end and two projections which swing outward and upward from the base of the cone to penetrate the cotyledons to within a short distance of their tips. The centre of the stele is composed of rows of slender cells. The length of these cells is from two to four times the width. The cells near the outer boundary of the stele (pericycle) are broader but not longer. The cells of the periblem are cubical and contain numerous plastids which at this time vary considerably in size. The dermatogen consists of several layers of smaller cubical cells. These also contain plastids which are here, however, of greatly reduced size.

When the embryo has reached about the twelve-celled stage an almost transparent layer suddenly appears over practically the entire ovule. External examination under the low-power microscope shows the surface of the ovule to be composed of the bulging, convex, outer walls of distended cells (Text-fig. 45). A section through such an ovule shows that the cells of the outer layer of the integument have absorbed water and increased greatly in size, and are almost cubical in shape (Text-figs. 43, 44). The growth of these cells does not take place at exactly the same time on every part of the ovule, hence it was possible in the same series of sections to find every stage of enlargement of these cells. In fact, there remains a small area near the inner free end of the ovule in which the cells do not, either now or later, participate in this enlargement. One week later these



F.A. Hallock *del.*

TEXT-FIGS. 35-49. 35. Longitudinal section of part of integument and nucellus of the age of that of Fig. 47. $\times 75$. 36. Embryo consisting of three tiers of actively dividing cells. Suspensor dividing in two planes (March 24). $\times 75$. 37. More advanced stage of above suspensor dividing in three planes (March 24). $\times 75$. 38. Still globular embryo (April 28). 39. Longitudinal section of embryo (June 16) and modified endosperm about it, diagrammatic. 40. Transverse section

large cubical cells divide periclinally so that the ovule is now covered by a double layer of large, almost transparent cells with large nuclei and highly vacuolated cytoplasm. The cells of the inner layer divide again by periclinal walls (Text-fig. 46). The cells of the outer layer do not undergo a corresponding division. Instead, they become greatly elongated radially to form huge tubular cells ten to fifteen times the original length of the cells before their elongation. These cells are delicate, thin-walled (Text-figs. 47, 49), closely packed, and wider towards the periphery of the ovule. The nuclei are large, but the cytoplasm is highly vacuolated. This structure does not fit the figures given by Baillon, but it may be remarked that his two figures (see p. 774) do not agree. This whole structure is now an exquisite thing when seen in entire longitudinal or transverse sections of the living ovule (Text-fig. 47).

On the contiguous faces of the paired ovules the same three layers are produced, but the outer cells elongate, here on one ovule and there on the other, so that in longitudinal sections the periphery of the ovule forms a waving line as the groups of elongated cells on one ovule lie opposite groups of shorter cells on the other. The small areas before mentioned, in which the cells do not enlarge, or divide, are now conspicuous. However, the cells surrounding these areas show the changes to a successively greater and greater degree, so that there is a transition between the cells which do and those which do not enlarge and divide.

This development of the outer layer of integument cells takes place not only in the maturing ovules, but also, strange to relate, in the small ovules in which the embryo sac did not develop beyond the seven-celled stage. After the outer walls of these undergo the development described above, the imperfect ovules are still somewhat smaller than the growing ones. They are colourless throughout, and lack all trace of the green endosperm. They are soft, easily flattened between thumb and finger, and seem to consist of nothing but integument and funiculus, while the growing ovules are now quite firm and resistant, with a plump filling of hard endosperm.

of a similar modified endosperm at level of the cotyledons. 41. Portion of one of the cotyledons of Fig. 40 showing cellular structure. 42. Longitudinal section of mature embryo (Sept. 16) showing boundaries of stele, periblem, and dermatogen. $\times 20$. 43. Part of a radial section through integument showing outer cells before enlargement (March 12). $\times 75$. 44. Part of a similar section of an older integument (March 24) showing beginning of elongation of outer cells of integument to form the ariloid structure. $\times 75$. 45. Surface view of group of outer cells of integument of stage shown in Fig. 44. $\times 75$. 46. Part of a radial section of a still older integument (March 31) showing appearance of periclinal walls in outer cells. $\times 75$. 47. Sagittal section through a fruit (June 16) showing embryo, modified endosperm surrounding embryo, storage endosperm with remains of antipodal mass at chalazal end, matured remains of integument consisting of smaller irregular cells within, and prismatic cells of ariloid structure without, the micropyle, the fibrovascular bundle of the raphe, the wall of the fruit, and the base of a style with persistent perianth. $\times 4$. 48. Detail of Fig. 47 showing longitudinal section through whole wall of fruit showing epidermal cells cutinized at surface and stone cells four layers deeper in. $\times 50$. 49. Detail of part of Fig. 47 showing two layers of crushed integument separating the persistent part of the latter from the endosperm. $\times 12$.

The cells covering the 'obturator' and the surface of the funiculus near the placenta also enlarge somewhat in the manner of those in the integument, but they remain globular. And though the cells of the integument may grow towards the cells of the 'obturator' and those of the upper funicle there is always a clear line of demarcation between the two regions (Text-fig. 47). The contents of these enlarged cells of the integument is at first colourless, but later a pale lavender colour develops, which slowly deepens until, in the ripe fruit, the contents of these outer cells of the integument has a deep wine purple colour. This 'pulp' remains as a permanent part of the seed. It dries down to a thin wrinkled membrane when the fruit becomes dried, but it expands again when wet to form a thick translucent capsule-like covering of the seed.

A longitudinal section through the fruit collected on June 16 shows well the structure of all its parts (Text-fig. 47). On the periphery of the section the ovary wall forms a large circle. The wall protrudes on one side to form the bases of the styles and on the other to form the pedicel of the fruit. Such a section through the ovary wall shows it to consist at this time of nine concentric rows of cells. The middle row is of thick-walled stone cells with deep pits. The four inner rows are composed of flattened cells varying somewhat in length. The four outer rows are composed of rounded, less regular cells, and the free surface of the outermost cells is strongly cutinized (Text-fig. 48).

The cut ends of the fibrovascular bundles appear at intervals in the path of the layer of stone cells, and the row here swings outward around the bundle in a semicircle. The outer layers of cells are consequently curved outward to form 'veins', which project to a greater or less degree on the surface of the fruit. Within this ovary there lie usually two ovules. Each of these now constitutes one half of a large, practically spherical mass which fills the cavity of the ovary. The section under discussion (Text-fig. 47) shows that a considerable portion of the bulk of the ovule consists of the large, delicate, radiating, club-shaped cells described above. The funiculus emerges from among these cells and attaches the ovule to the placenta. The ends of these cells are flattened where they meet the ovary wall, and are bevelled where they dovetail with the ends of similar cells from the adjacent ovule. The inner ends of these cells rest against the cubical cells described above. Below the cubical cells are two to four layers of smaller cubical cells, and below the placenta the micropylar canal is still indicated by a dark band of small cells extending through these inner layers of cells to the boundary of the endosperm.

Two further layers separate these smaller cubical cells from the mass of endosperm (Text-fig. 49). These two layers are composed of the collapsed walls of integument cells that have been crushed back by the growth of the endosperm. The two layers are of similar structure, but react

differently with Delafield's haematoxylin. The outer layer takes a deep blue colour, the inner remains entirely unstained. The two layers are usually contiguous, but frequently they are separated by several layers of cells which have almost, but not quite, collapsed. The large fibrovascular bundle of the funiculus stands now directly against the inner layer of crushed cell walls. When the outer layer approaches the bundle it diverges from the inner, swings out, and forms a semicircle around the bundle. The origin of the inner layer of crushed cell walls is evident, and has already been described. The outer layer is probably formed by a crushing and folding in the outer tissue of the integument caused by the expansion of the central portion of the ovule. These two layers of collapsed cell walls inclose the large, almost spherical mass of endosperm cells, which are now filled with starch. The outermost cells of the endosperm are elongated, with their long axes lying perpendicular to the surrounding wall-layers. The inner cells, on the other hand, are irregular, angled, and closely packed. The embryo lies in the endosperm in a region just below the micropyle. The radicle points towards the micropyle, and the cotyledons away from it. Immediately enveloping the embryo is the elongated mass of liquid-filled, partially degenerated endosperm cells described above.

Between June and September the fruit undergoes little change. The outer cells of the integument are filled with a purple 'juice'; the mass of endosperm cells has now become extremely hard. The embryo is mature, and the inner endosperm, which immediately surrounds it, is now hard but rather translucent, and the cells are still devoid of starch. The ovary wall thickens somewhat, and later gradually becomes dry, fragile, and about the thickness of a dried oak leaf. Green (23) described the fruit of *G. elliptica* as irregularly dehiscent. Brandegee (9) objected to this statement, and described the fruit as indehiscent. The following observations seem to confirm the latter opinion.

No abscission layer forms to cut the fruit cluster from the tree. Unless the fruit clusters are removed by animals they remain firmly attached, even when emptied of seeds, until they are so brittle and dry that they are shed, as dead branches are shed, when the tree is lashed by driving storms.

An examination of these persistent catkins showed that the emptied fruit wall, which occurs on exposed parts of the tree, invariably shows a small irregular opening in the apex of the wall. Occasionally the walls are filled with the castings of a small larval insect which has eaten the seeds. In these cases some of the walls show the same apical opening, while others show no opening at all except the minute lateral pinhole bored by the larva in its escape from the fruit.

In the case of poorly pollinated catkins on which only an occasional fruit is matured, or in the case of well-developed fruits produced within the protection of dense tufts of stiff twigs, the wall of the fruit shows no opening

at all, even in February. Finally, in the case of catkins which compose the large hoards collected by wood-rats or other rodents, the opening in the fruit wall is very large—sometimes practically the entire wall is missing.

These facts seem to indicate, then, that the fruit of *G. elliptica* is naturally indehiscent, and that the size of the opening found in the wall depends on the particular animal that makes the opening.

The following table gives the date for each phase of development of the staminate and pistillate flowers from October 29, 1924, until September 16, 1925. Some variation in details would, of course, result from different weather conditions, and, as Setchell (40) shows, from the altitude at which the plants grow, however, the general trend of development would remain constant. The date of fixation was two to four days later than the date of collection here given.

Propagation.

At the present time the artificial propagation of *G. elliptica* seems to be accomplished chiefly by cuttings. No records have been found of any germination of the seeds. Lindley (35) states only that the cotyledons remain permanently anchored in the seed as in *Quercus*. He does not state whether this conclusion was drawn from actual observations of germinating seeds or from a study of the structure of the seed. Lubbock's statement (1892) is ambiguous. McMinn in a letter (1925) reports that he was unable at any season to find seedlings of *G. elliptica*. The writer searched persistently among scores of fruiting plants with the same result, even though fertile seeds are abundantly produced and can be unmistakably distinguished from the unfertilized ovules. Certain horticultural dictionaries omit all reference to seeds as a possible means of propagation in this plant.

Whether the failure of the seeds to produce plants is due to the fact that few seeds escape the wild birds and other animals of this region, where encroaching civilization is decreasing the feeding-grounds of these creatures and consequently increasing the competition for food among them, or whether it is due to a change in the climatic conditions of this region, is at present unknown.

In the canyons, where *Garrya* is native, propagation is occasionally effected by the wood-rat, *Neotoma fuscipes annecteus*, Elliott. The type of habitat of this species of *Garrya* and of this wood-rat are identical, and the tree furnishes a considerable portion of the animal's food. In the region studied it was difficult to find a considerable tract of *G. elliptica* which did not also harbour one or several wood-rat nests. These animals are large and strong. According to Goldman they average 43 cm. in total length, with a body length of 22 cm. They build conical nests of rather loosely piled sticks somewhat horizontally arranged at the base, more or less vertically placed at the apex. Three unusually large nests which were measured

SEQUENCE OF STAGES IN DEVELOPMENT OF FLOWERS.

Date.	Stage of Pistillate Flower and Fruit.	Stage of Staminate Flower.
Oct. 29	Nucellus forming.	Sporogenous cells all alike.
Nov. 14	Primary archesporium formed. Definitive archesporium and tapetal cells separated.	Flowers begin to show above bracts. Tapetum and sporogenous cells differentiated. Pollen mother-cells formed in some flowers.
Nov. 28	First division of definitive archesporium has occurred (rarely the second).	Flowers lifted by pedicel completely above bracts. Tetrads and ripe pollen grains formed. Anthesis begun.
Dec. 12	Styles begin to push out from behind bracts. Row of four potential megaspores completed.	Anthesis and shedding of pollen in general.
Dec. 20	Germination of inner megaspore into an eight nucleate embryo sac.	
Jan. 2	Style withered. Embryo sac enlarged. Lateral nucellar tissue partly absorbed.	
Jan. 16	Sac more enlarged. Lateral nucellar tissue completely absorbed.	
Jan. 30	Ovary wall begins to appear above bract.	
Feb. 5	Divisions of endosperm nucleus begun. Each antipodal divides into two or four cells.	
Feb. 14	Fifty endosperm cells in one longitudinal section of sac. Antipodals in same section deeply stained.	
Feb. 28	Half of ovary visible above bract. Endosperm mass greatly enlarged. Antipodal cells vacuolate, staining faintly. Endosperm cells are dark.	
Mar. 12	Proembryo of two cells.	
Mar. 24	Proembryo of three to fourteen cells. Enlargement of outer cells of integument initiated.	
Mar. 31	Periclinal wall present in each arilloid cell of integument. Embryo of seventy-two or more cells.	
Apr. 14	Arilloid structure complete.	
Apr. 28	Embryo 15 cells in diameter, 16 cells long.	
May 20	Cotyledons first evident.	
June 16	Cotyledons 7-10 cells wide and 20-30 cells long.	
Sept. 16	Flesh purple; seed ripe.	

averaged $3\frac{1}{2}$ ft. basal diameter and $4\frac{1}{2}$ ft. in height. Each of these nests included in its structure the middle portion of several living, lower, horizontal limbs of *G. elliptica* under which it stood. Each nest had several entrances both from above and below ground. Entrances invariably opened into the living limbs around which the nests had been built.

The propagation and dispersal of this *Garrya* is often aided by the wood-rat. Besides feeding on the seeds of *G. elliptica* the animal habitually cuts off young terminal branches about 6 to 14 inches long, including two or three years' growth, drops these to the ground, and then collects them into a heap. The stems are then devoured while the leaves and terminal buds are left in a little pile. This extensive pruning by the wood-rat accounts in part at least for the shrublike appearance of many of the younger plants of *Garrya*. Fallen twigs cut off by the animals are frequently overlooked by them. If these are dropped at a time when conditions favour growth, and are covered by sliding earth, several large white rootlets develop at one of the nodes, soon the stem turns upward, and a new plant is started. Five plantlets found in the region studied clearly had this origin. The other young plants seen were too old to reveal their exact origin.

One example of layering was seen. The lowest branch of a shrublike plant had been held to the soil by a sliding stone. This branch had rooted in the manner described above, though still remaining attached to the parent plant. However, the natural propagation of *G. elliptica* in the region studied seems to be chiefly effected by the feeding habits of the wood-rat, *Neotoma fuscipes annectens*.

Germination of Seeds.

The germination of the seeds of *G. elliptica* and the method used to induce germination are described in a separate paper which is to follow; but a few facts are noted here as they bear somewhat on the present work.

The radicle emerges from the seed and turns toward the substratum, the hypocotyl forms a horseshoe-shaped arch with the loop pushing above the soil, the cotyledons are drawn from the seed coat when the food supply in the endosperm is exhausted, and they persist as broad flattened photosynthetic structures with the plumule between them developing slowly.

IV. DISCUSSION.

Of the many views expressed concerning the phylogenetic position of the Garryaceae, two are predominantly current in the literature of to-day. It is a curious fact that these represent the two most extreme opinions that have been expressed during the whole history of the study of the Garryaceae. The two current views are that of Engler and Gilg (20), which places the Garryaceae among the most primitive of Dicotyledons, and that of

Wangerin (44) (in Engler, 'Pflanzenreich', 1910), which places them as the highest of the Archichlamydeae under the order Cornaceae.

It is, therefore, necessary first to determine which of these diametrically opposite views is supported by the new evidence here offered and then to determine, if possible, the position of the Garryaceae among the higher or lower Dicotyledons as the case may be. Schürhoff points out the frequency with which similarities in anatomical features accompany similarities in morphological and cytological structures. On the other hand, Engler and Gilg warn that genetic relationship can frequently be proved to exist between plants which are extremely dissimilar externally where these differences may be due to climatic conditions. The external, gross characters are therefore of value in classification within certain limits, but finally an appeal must also be made to the morphological evidence of genetic relationship.

The first question to be considered is whether the known structural and developmental characters of *G. elliptica* are those more common in primitive or in advanced Dicotyledons. The characters of the Garryales enumerated by Engler and Gilg are as follows: Flowers unisexual and haplochlamydeous. ♂ Flower—perianth parts four. Stamens four alternating with the perianth parts. ♀ Flowers naked, carpels two to three. Ovary superior, one-celled with two ovules each pendent from a parietal placenta. Integument complete or incomplete. Fruit with a thin pericarp; seeds one to two with a thick flesh seed coat. Embryo small in the tip of the fleshy albumen. Plant with four angled branches and opposite lanceolate evergreen leaves. Flowers in catkin-like panicles.

Many of the characters listed above are frequently found in primitive seed plants. These are attributed to *Garrya*, however, on the basis of earlier work, and are, in many cases, attributes concerning which different workers on the genus disagreed. Hence it is necessary to examine the characters assigned to *Garrya* by Engler in the light of the present research in order to determine the correctness of the description, and hence of the relationship there assigned to it.

In the first place the flowers are as described definitely unisexual. But this condition in *G. elliptica* differs somewhat from that found in such primitive plants as *Morus alba*, which, according to Schaffner, shows 21 per cent. of the plants as 'intermediates', and *Salix amygdaloides*, which shows almost 10 per cent. of 'intermediates'. In the intermediates of the latter the catkins produce pistillate flowers above, staminate flowers below, and frequent abnormalities in the transition zone. No intermediates were found in *G. elliptica*, though they were sought for throughout the course of the investigation. Dioecism seems to be in a more fixed, less plastic condition here than in the plants generally accepted as primitive. Furthermore, dioecism is not confined to primitive seed plants for, as is well known, dioecious flowers are not rare even among the Compositae, nor are monoecious and

hermaphrodite flowers wanting among the primitive genera. The evidence from this quarter therefore favours the view that *Garrya* is primitive rather than the contrary, but is not at all conclusive.

The staminate flowers are indeed haplochlamydeous, but, according to the reports of Baillon (3), the exactness of whose observations is unquestioned, this haplochlamydeous condition results from the reduction of the sepals, the primordia of which are clearly and invariably to be seen in the early formative stage of the staminate flower.

Engler and Gilg's characterization of pistillate flowers as naked is, it is true, in accord with Baillon's published conclusions but not with his descriptions. Baillon, as has been noted above, reported two circles of alternate bractlets crowning the ovary, the members of the outer circle dense and green, those of the inner circle often expanded and petal-like. The evidence offered by the fibrovascular bundles running into these 'folioles' and the differences exhibited between the inner and outer circles, the definite position of these structures with relation to each other and to the styles favour the view of Harms (1898), and of the still earlier workers, that these structures represent reduced perianth parts, and that the pistillate flowers cannot therefore be considered as 'naked'. These same evidences would indicate also that both sepals and petals are present though in an exceedingly reduced form, and the presence of petal-like structures in the staminate flower tends to support this view. A further deduction which is inevitable from this theory is that the ovary is, as has been stated by the majority of workers, distinctly inferior. The flower parts are therefore epigynous. The number of parts in the staminate flower is small and definite; that of the pistillate flower is smaller and less definite, but this can be accounted for by the very clear evidences of reduction.

The flowers of *G. elliptica* are arranged in catkins, but these catkins are strikingly different from those generally found among primitive plants. The flowers are definitely opposite and distinctly four-ranked, while in the catkins of primitive plants the flowers are almost always arranged either spirally or irregularly. *Garrya gracilis*, Wangerin, as figured by Wangerin, produces its pistillate flowers in the axils of large, opposite, distinctly leaf-like structures, the decussate pairs of which are widely separated on the elongated stem. In *Garrya Wrightii*, Torr. the length of the fertile portion of the stem is reduced, the opposite pairs of subtending bracts are considerably less leaf-like, and the lower fruiting branches are drooping. It is probable that the dense pistillate catkins of *G. elliptica* represent a still further reduction of the leafy flowering branches. The droop of the flowering pistillate branches of *G. gracilis* and *G. Wrightii* is apparently due to the weight of the fruits, combined with the slender delicacy of the branchlets. The oblique position assumed by the rigid pistillate catkins of *G. elliptica*, in even the youngest stages, may be a relic of the drooping character of the

less reduced species of *Garrrya*, or it may be a newly acquired arrangement by which the catkins are held free from the closely clustering leaves. However this may be, the droop of the pistillate catkins is clearly independent of gravity, and is the result of a distinct bend in the stiff, rigid catkin axis.

Lastly, the integument is reported by Engler and Gilg as complete or incomplete. The integument of *G. elliptica* is doubtless the one feature of this species concerning which there has been the greatest misconception. The reports of the various workers indicate that Baillon alone studied the fresh pistillate flowers, and then only for a limited range of development. In the dried fruits the enlarged outer cells of the integument invariably dry down to form a thin layer over the surface of the seed. This layer is frequently retracted somewhat in the region of the micropyle and resembles, in the dry fruit, an incomplete integument. The integument is, however, as has been stated, single and exceedingly thick and massive until it is finally absorbed by the germinating seed, and the structure which is described by Engler and Gilg as a thick fleshy seed coat consists simply of the curiously developed outer cell-layers of integument described above. While the absence or incomplete development of the integument is generally accepted as a character marking exceedingly primitive plants, Engler and Gilg, Coulter and Chamberlain, and others state definitely that a single massive integument, with a small nucellus, is a character found typically among the highest Dicotyledons, being distinctly characteristic, e. g., of the Umbelliferae and Sympetalae. Coulter and Chamberlain state regarding this that though the single massive integument is found in a few species of Ranunculaceae, Leguminosae, &c., it is a constant characteristic of the ovules of Umbelliferae and Sympetalae. 'There seems to be,' they add, 'every indication that the two integuments are characteristic of the ovules of the more primitive angiosperms; that they persist among Monocotyledons of even the most highly specialized families; but that among Dicotyledons they are replaced in the higher groups by a *single massive integument*. The fact that the single integument is more massive even than both integuments together, when there are two, suggests that it represents two integuments in the sense that their primordia are no longer differentiated. This is very far from meaning that two integuments have fused to form the single one, but that a single integument is developed by the same region that in other cases produces two.' Moreover, the writer has been unable, so far, to find any report of an exceedingly primitive plant with a single massive integument and a small nucellus. This seems then, accepting the standards of classification as presented by Engler, a character of definite rather than of merely relative diagnostic value. This examination of Engler and Gilg's description shows that while none of the characters recorded there would conclusively place *G. elliptica* among the primitive

Dicotyledons, the single massive integument with a small nucellus clearly indicates an alliance with more advanced seed plants.

Among other characteristics not mentioned by Engler and Gilg, and which might indicate that *Garrya* is a primitive type, is the probably anemophilous habit, suggested by the pollen grains which have the sculptured exine characteristic of anemophilous plants, and confirmed by such observations as were made in the field. This seems, however, to be a secondary adaptation to environment. Such retrogressive or atavistic adaptations are not rare among plants. Striking examples of these are afforded by *Cyclamen*, *Bartsia*, and *Calluna vulgaris*, which, according to Knuth, are insect-pollinated in the early part of the season but later become adapted to anemophily; and by the ash which, although closely related to typical entomophilous flowers, has adapted itself entirely to an anemophilous habit.

Another character which is also suggestive rather than conclusive evidence of primitiveness is the presence of a woody stem. While a woody stem is in general to be regarded as a primitive feature, yet the exceptions are numerous enough to indicate that no absolute diagnostic value can be assigned to it. Woody shrubs such as *Baccharis halimifolia*, *Artemisia*, *Senecio*, and even trees like *Vernonia*, are by no means rare, even among the Compositae and many other families of higher Dicotyledons. Furthermore, the wood of *G. elliptica* is 'imperfect', suggesting again an adaptation to environment.

Among the other morphological characters of *Garrya* which should be mentioned here are many which occur, not only among more primitive angiosperms but also in higher ones. These characters, therefore, have as yet no definite diagnostic value for the distinction of these types. Such characters, e. g., are the small embryo in the copious albumen; the elongated egg (oospore); the active antipodal derivatives; the formation of an elongated suspensor; the undeveloped state of the embryo in the mature seed.

However, the single primary archesporial cell in the ovule and the suppression of the parietal tissue are predominantly features of higher dicots rather than of lower ones. The formation also of a long row of four potential megaspores, of which the innermost develops into the embryo sac is a definite character of the Sympetalae, and is, therefore, of distinct diagnostic value. The anatropous ovule is found among high and intermediate dicots, but seldom if ever among the very primitive ones. The eight-nucleate embryo sac of *Garrya* shows none of the interesting variations so frequently exhibited by the embryo sacs of angiosperms sometimes regarded as primitive, such as *Peperomia* (Campbell, 1899; Johnson, 1900, 1907), but is instead of the type most commonly found among angiosperms of intermediate and higher rank.

The preceding discussion shows in brief that while many of the characters of *G. elliptica* are those associated with primitive Dicotyledons

these are not confined to primitive ones. Certain other characters of *Garrya*, on the other hand, such as epigyny, cyclic arrangement of the parts of the flower, and likewise the single massive integument, the row of four megaspores and the germination of the inner one, are characters found only in higher Dicotyledons. The balance of evidence, therefore, would definitely indicate that *G. elliptica* belongs among the higher orders of Dicotyledons.

A consideration of Engler and Prantl's classification (which places the Garryaceae among the Umbelliflorae, regarded as the highest of the Archichlamydeae) necessitates a review of certain facts already mentioned. According to Engler and Gilg (20, pp. 308-9), the distinctive characters of the Umbelliferae are epigyny, cyclic stamens, reduced number of carpels, sepals mostly reduced in size, and a simple, definite floral formulae, ovules one or two, a single integument, and a fleshy endosperm. Coulter and Chamberlain (p. 251) state that 'the Umbelliflorae present the same combination of characters as those that belong to the Sympetalae, excepting sympetaly', and that in regard to this character both groups show exceptions.

A comparison of characters of *G. elliptica* with those just mentioned brings out marked resemblances of it to each of these two groups. The character of epigyny has already been fully discussed, and the cyclic arrangement of the stamens is a fact of common knowledge. The reduced number of carpels is evident, whether the ovaries possess two styles or (as they may rarely) three styles; and the reduction in the sepals is so evident as to need no further discussion. The floral formula of the staminate flower is simple and perfectly clear, but that of the pistillate flower is less definite. Here the number of reduced sepals varies. When two are found they are usually about equal and less reduced in size; where three are present the size varies considerably in the same flower, one being always noticeably smaller than the other two and rather irregular in form. The same is true of the petals. The structures around the base of the stigma are, however, difficult to interpret positively. Even when most regularly formed there are besides the two to three definite rudiments of both sepals and petals minute hard irregular projections, the morphological nature of which cannot with our present knowledge be stated with any degree of certainty. However, three sepals have been definitely observed, and there are sometimes three other structures alternating with these which may with some justification be termed petals. The staminate flower, as has been said, is cyclic and definite in plan. Hence, since it is not conceivable that the staminate and pistillate flowers of the same species are so widely separated in evolutionary position that the one is primitive and the other highly specialized, the only logical conclusion is that the pistillate flower also is definite in its formula, and the absence of certain parts must be attributed to reduction.

Such a reduction of these floral parts could justly be regarded as an

adaptation to an environment in which such organs would be not only useless but wasteful for a plant in which, as is apparently true in *Garrya*, entomophily has been discarded. Moreover, the hard subtending bracts of the catkins are so placed as to offer abundant protection to the flowers in their early stages, while the dense hairs and strong wall of the ovary afford sufficient protection to the mature pistillate flower. The retention of the fully developed petals in the staminate flower would be advantageous since the delicate thin-walled microsporangia require protection against drought, &c., up to the very shedding of the pollen, and stamens lacking this protection could seldom if ever mature.

The further characters mentioned by Coulter and Chamberlain, namely, the single massive integument and the very small nucellus, have been fully considered above. These features imply then that, except for the distinct floral parts, *G. elliptica* could be placed equally well with the Umbelliflorae or the Sympetalae. However, the formation of a row of four potential megaspores and the germination of the inner one to form the embryo sac are especially characteristic of the Sympetalae.

The peculiar development of the outer cell layers of the integument has, at present, no real value as an index of relationship since, as far as the writer was able to determine, no Dicotyledon mentioned in botanical literature offers a parallel to this. Nor is it closely comparable with the fleshy integuments of Cycads and Ginkgo. It must be remembered, however, that the precise origin of the pulp in most fruits has not yet been investigated, and it is entirely possible that future studies may give phylogenetic significance to this phenomenon. Baillon has designated the structure in question as an 'arille généralisé'. Whether a fleshy coat arising in this way can be called an arillus depends entirely upon the definition accepted for this term, for the term 'arillus' has as yet no morphologically precise and generally accepted meaning, as may be seen in the differing definitions of the word presented by Schneider, Gray, and B. Daydon-Jackson. We will, therefore, call the structure under discussion simply an arilloid development of the outer cell layers of the integument arising, directly or indirectly, in consequence of fertilization.

Many of the characters of *G. elliptica* then are also found in the Umbelliferae. Many other characters, such as the row of four potential megaspores and the germination of the inner to form the embryo sac, ally it with the Sympetalae.

The principal structural difference between *G. elliptica* and the accepted genera of the Cornaceae is the unilocular ovary of the former. Baillon considers it probable that the ovary of *Garrya* is a two-loculed structure in which the placentae have not developed far enough to divide the cavity into two parts. This behaviour would not be inconsistent in a species which shows such general and marked reduction.

As a whole, then, the locating of *G. elliptica*, and the other species of this genus, among the Dicotyledons, presents three possibilities: first, to accept Wangerin's classification and place the genus in the Cornaceae, the highest of the Archichlamydeae; secondly, to transfer the order Garryales to a position between the Umbelliflorae and Sympetalae as a transitional group, because of the distinct petals on the one hand and the structure and development of the potential megaspore on the other. This last character suggests the third possibility, the classification of this group as another poly-petalous family of the Sympetalae.

Two objections appear which are equally applicable to the last two possibilities, first, that only one species of *Garrya* has been intensively studied, and it is not impossible that this species may show characters which are atypical for the genus; and second, the development of the flowers of the Cornaceae is so little known that no sure grounds exist at present on which to justify a separation of the Garryaceae from the Cornaceae. The author, therefore, holds that until further investigation is made of the development of other Garryaceae and of the Cornaceae, the position chosen by Engler (1910) for the Garryaceae (namely, as the highest of the Archichlamydeae immediately preceding the Sympetalae) is, of the three possibilities suggested above, the safest and the one most adequately justified by the evidence available.

V. SUMMARY AND CONCLUSIONS.

1. The 'folioles' of *G. elliptica* are considered as epigynous sepals and petals because of their internal microscopic, as well as external, structure and their positions with relation to each other, to the styles, and to the ovary.

2. The ovule does not possess an incomplete integument or two thin integuments, as has been most generally reported, but a single massive integument around a small nucellus. Sometime after fertilization the outermost layer of integument cells swells around the whole seed. Each cell elongates enormously radially to form the extensive 'flesh' of the ripe fruit.

3. The single primary archesporial cell divides to form a single tapetal cell and one definitive archesporial cell. The latter divides twice to form a longitudinal row of four potential megaspores, and the inner one of these develops to form a typical seven-nucleated embryo sac. The embryo sac enlarges, while the double layer of nucellar cells on each side of it entirely disappears.

4. The endosperm nucleus divides, and a large mass of innumerable endosperm cells is formed before the egg-cell divides. The endosperm nuclei scatter throughout the embryo sac, and walls are formed almost immediately. The first three divisions of the long, 'vermiform' fertilized

egg are transverse, followed by one longitudinal division. No hypophysis is formed, but the tip of the radicle is covered by the terminal cells of the long filamentous suspensor, otherwise the embryo follows the *Capsella* type of development, though the differentiation of the cotyledons is delayed until the spherical embryo is greatly enlarged. The mature embryo possesses no plumule and no root cap. The stele is definitely outlined, but the vascular tissue is still undifferentiated.

5. Epigyny, cyclic stamens, reduced number of parts, reduced sepals, simply floral formula, and character of integument exclude *G. elliptica* from the primitive position assigned to it by Engler and Gilg (1924). These same characters associate it with the Umbellales.

6. The character and development of the potential megaspores associate it also with the Sympetalae. The evidence would suggest, therefore, that *G. elliptica* belongs between these two groups. Many morphological features associate the plant with the Cornaceae, the highest of the Umbelliferae. Therefore, until the developmental characters of the Cornaceae are determined and other species of *Garrrya* are investigated, it seems wise to accept the classification of Wangerin in Engler (1910) and place the Garryaceae as the highest of the Umbelliflorae immediately preceding the Sympetalae.

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EXPLANATION OF PLATE XXXVI.

Illustrating Miss F. A. Hallock's paper on The Relationship of *Garrya*.

- Fig. 1. Photograph of a tree showing tassel-like clusters of staminate catkins (Dec. 12, 1925).
- Fig. 2. Portion of recumbent trunk of same tree seven inches in diameter at point *x*.
- Fig. 3. Branch from pistillate tree showing characteristic position of growing female catkins (Dec. 12).
- Fig. 4. Cluster of staminate catkins (Oct. 29). $\times \frac{1}{2}$.
- Fig. 5. Pistillate catkins (Oct. 29). $\times \frac{1}{2}$.
- Fig. 6. Staminate catkins (Nov. 14) showing male flowers emerging from behind bract. $\times \frac{3}{8}$.
- Fig. 7. Pistillate catkin (Nov. 28) showing four-angled prismatic form. $\times \frac{1}{4}$.
- Fig. 8. Pistillate catkin (Dec. 12) showing styles emerging from behind bracts. $\times \frac{1}{4}$.
- Fig. 9. Cluster of pistillate catkins (Dec. 20) showing marked increase in length.

Figs. 10-13. Pistillate catkins (Jan. 2, 1925) showing long prismatic form and withering of styles.

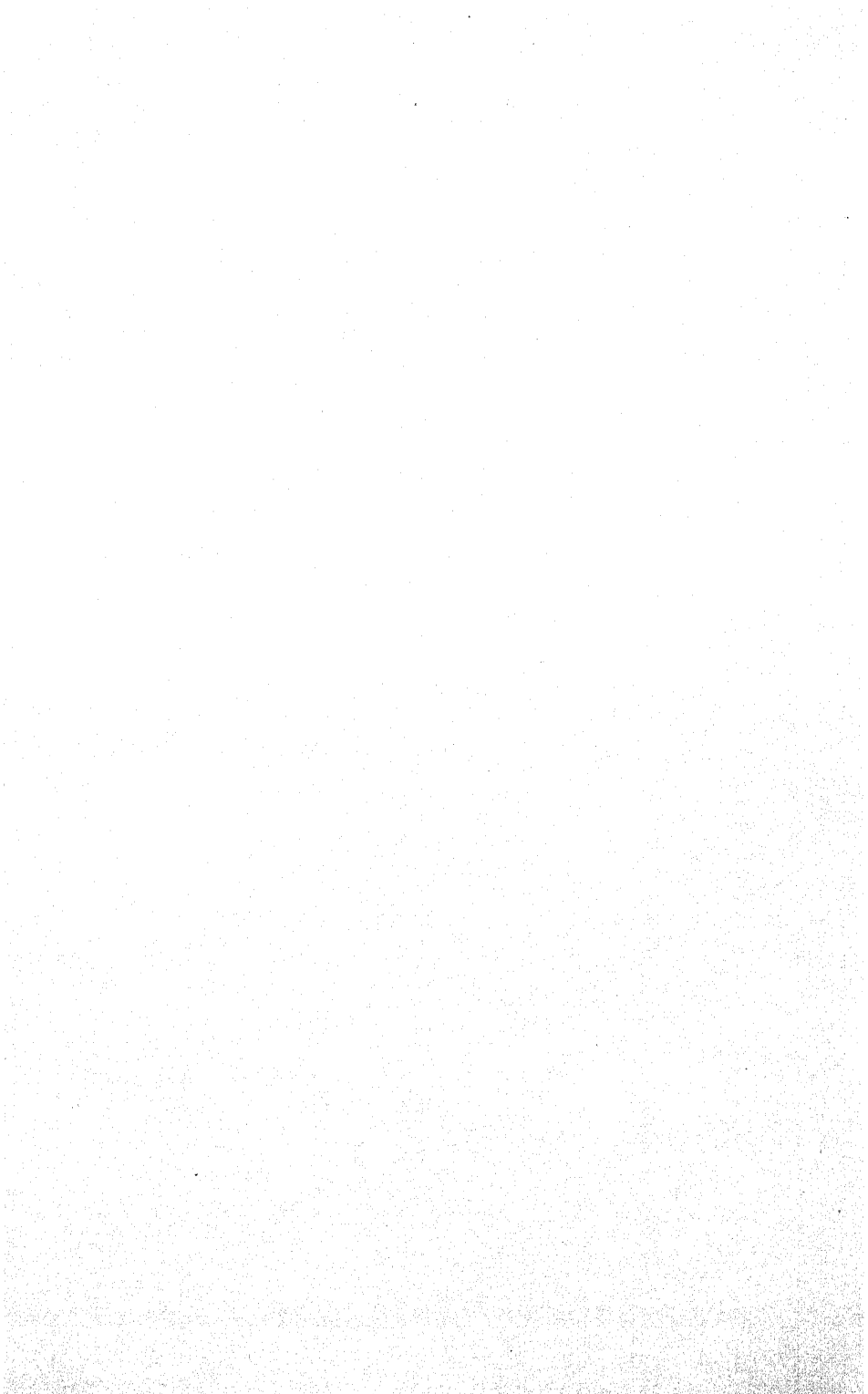
Fig. 12. Cluster of pistillate catkins (Jan. 30) showing tips of ovaries appearing above bracts.

Figs. 13, 14. Older pistillate catkin (Feb. 28) with tomentose ovaries visible for half their lengths above bracts and perianth parts at base of styles easily visible in many flowers as at *x*. Note three styles in Figs. 14 and 16 at *y*.

Figs. 15, 16. Pistillate catkin (March 12) showing rapid growth of ovaries, more nearly transverse position of bracts, and dense tomentum.

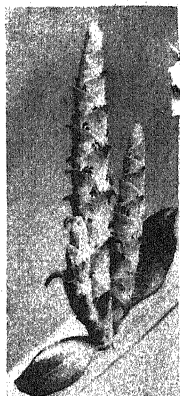
Fig. 17. Pistillate catkin (March 24) showing perianth parts at *x*.

Fig. 18. Pistillate catkin (May 25) showing hairy pericarp of full grown fruit, embryo not yet mature (perianth parts easily visible). $\times \frac{3}{8}$.





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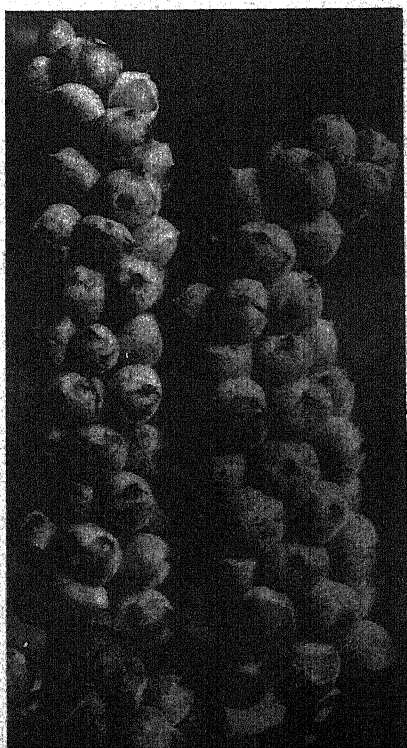
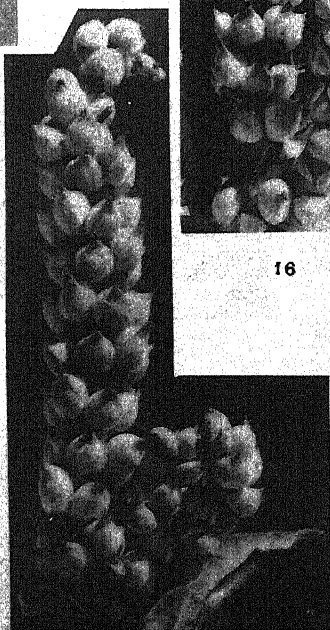
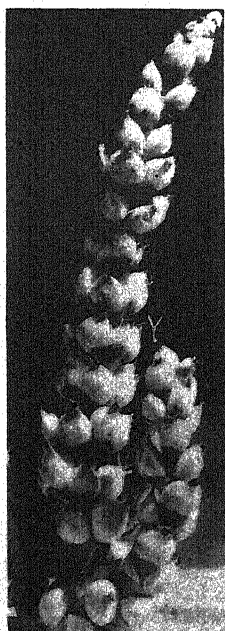
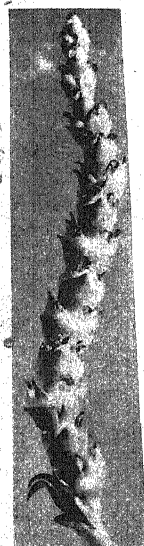
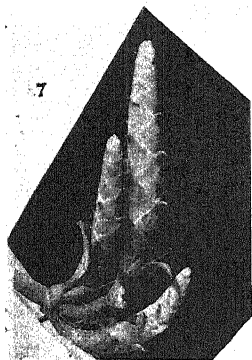


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8





On *Arceuthobium pusillum*, Peck.

II. Flowers and Fruit.

BY

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AND

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With eleven Figures in the Text.

PREVIOUS accounts of the reproductive sequence in *Arceuthobium* have been based upon study of the mature fruit of *A. oxycedri* (5, 3) and *A. occidentale* (8), and of the mature flowers of *A. oxycedri* and a few stages in their development (5, 4). The development of the fruit has not hitherto been described for any species, and some of its features are difficult to interpret correctly without a knowledge of intermediate stages.

The additional material sent us by Professor Faull, collected in June and July, has enabled us to piece together a fairly reliable connected outline of the main facts for *A. pusillum*. In this we have been much indebted to Miss A. J. Davey, M.Sc., who kindly prepared some series of microtomed sections and in other ways assisted us from time to time.

THE MALE FLOWER.

The male flowers we have examined in bud, in material collected on August 12. They agree very closely with the descriptions of other species, but at this stage there has been little intercalary elongation below the anthers (cf. Johnson, 5), which have therefore not been carried up 'on to' the perianth segments (Fig. 1, A, B). The anther is kidney-shaped in face view, with a central depression.

According to Heinricher (4) the anther of *A. oxycedri* presents some unique features. It has a single annular pollen-sac round a central sterile column, and the mature wall is of one layer of cells only, the fibrous layer, though with traces of a second. Heinricher's pupil, Pisek (10) has confirmed

that the archesporium is annular, and found a second wall layer between the epidermis (the exothecium) and the tapetum.

In our material the archesporial tissue is still intact, the cells adhering to one another. From a careful study of a microtome series we can fully

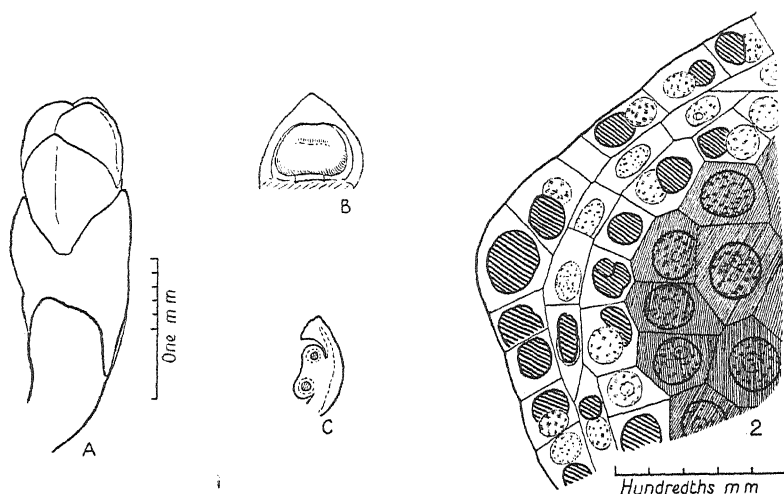


FIG. 1. *Arceuthobium pusillum* ♂. A, entire shoot with terminal trimerous ♂ flower, August 12. B, perianth segment with adnate anther. C, longitudinal section of same; sporogenous tissue shaded.

FIG. 2. Portion of anther in transverse section of flower, showing tapetum and two other layers of cells outside the sporogenous tissue (shaded).

confirm that in *A. pusillum* also the archesporial tissue is continuous right round the central 'columella'. Surrounding it is a layer of tapetal cells, as described by Heinricher, and outside this are two layers of cells, an inner of shallow cells and an outer of larger (deeper) cells (Fig. 2). If the latter becomes the fibrous layer, as Pisek implies, *Arceuthobium*, as Heinricher points out, is a solitary exception among the angiosperms, in respect of its epidermal fibrous layer as well as of its annular pollen-sac.

THE FEMALE FLOWER AND FRUIT.

The Female Flower Bud in August.

In the third season of growth of the host-twigg and the endophytic system the aerial shoots emerge and the flowers develop. At the beginning of July the female flowers have not yet been formed (Fig. 3). By the middle of August all the essential parts are present in the bud (Fig. 4). The perianth, of two opposite segments, connate below, is still closed over the style. The perianth segments of the terminal flower alternate with the leaves of the whorl below; those of axillary flowers without leaves on their own stalks, are placed laterally (cf. 11, Fig. 1). The outer epidermis

has a very thick cuticle and a reddish purple pigment; stomata are present with transverse pores, as in the leaves.

Two vascular bundles enter the base of the flower, each passing up into a perianth segment, branching in a fan-like manner, and reaching

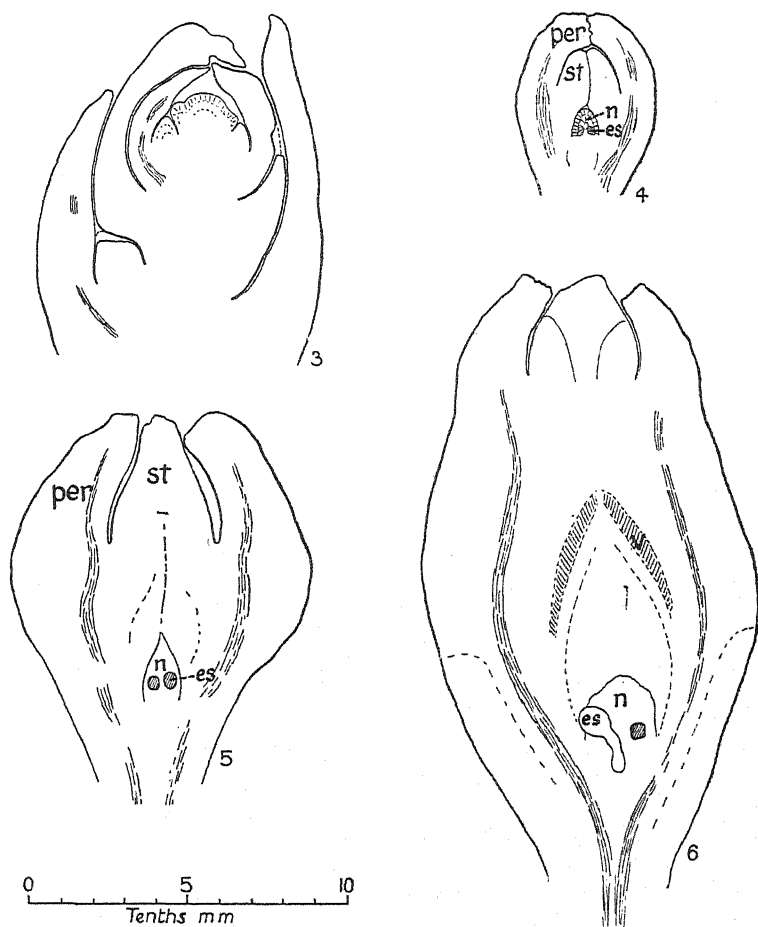


FIG. 3. *Arceuthobium* ♀. Young bud, July 4 in first year of emergence, slightly oblique longitudinal section, median at the apex. The flower bud has not yet been initiated.

FIG. 4. Flower bud, middle of August, longitudinal section in median plane of the perianth segments, *per*; *st*, style; *n*, nipple with two embryo-sacs (? or archesporial cells) *es*. (Cf. Fig. 7)

FIG. 5. Pollinated flower in June, longitudinal section, as lettering Fig. 4.

FIG. 6. Young fruit, July 4: *es*, fertile embryo-sac with basal haustorium, grown out of the nipple, *n*; the second, abortive embryo-sac is shaded; *v*, young viscin tissue. (Cf. Fig. 8).

nearly to the tip. At this stage the bundles are only meristematic, and stand out conspicuously from the surrounding parenchyma, the cells of which are larger, and already contain characteristic gelatinous masses of resistant material staining deeply with safranin.

The ovary is inferior and unilocular. Running from the cavity of the ovary throughout the length of the style is a stylar canal, lined by a thinly cuticled epidermis. In transverse section it is a narrow, sometimes tri-radiate slit.

Rising from the base of the ovary and filling its cavity is a dome of tissue called by Johnson (5) the 'ovarian papilla'. A corresponding structure in *Loranthus* (*Elytranthe*) *sphaerocarpus* has been called by Treub (12) the 'mamelon' or nipple. In *Arceuthobium* it is not lobed, as in Treub's plant, nor does it anywhere adhere to the surrounding ovary wall. This structure has commonly been regarded as a placenta, yet the application of this term is scarcely justified by the relatively undifferentiated state of the structure. While it differs in form and size from the shoot apex of an earlier stage (cf. Figs. 3 and 4), it compares well enough with the smaller axillary growing points. Regarded from a causal point of view, it may partake of the nature of both stem apex and placenta, and we prefer to use a non-committal term. Johnson's term 'papilla' seems scarcely appropriate to a multicellular dome, and we therefore adopt Treub's term, 'nipple'.

On opposite sides of this nipple in the plane of the perianth segments, two hypodermal cells are distinguishable by their larger size and by the larger size and more open structure of their nuclei (Fig. 7). They are, in all probability, the embryo-sacs already differentiated.¹ A similar stage has been figured for *A. oxycedri* by Johnson, who states that each embryo-sac represents the lower of two daughter cells formed by the division of a hypodermal archesporial cell (5, Pl. X, Fig. 5).

The Pollinated Flower in June.

By the following June the flowers have opened and have already been pollinated. In most flowers the stigmas have shrivelled, but some still bear slender septate hairs. The superficial stigmatic region of very small cells contrasts very sharply with the large-celled parenchyma that forms the bulk of the style. Among the stigmatic hairs are occasionally to be found round spiny-coated pollen grains.

The stigma is often bifid, but scarcely less often trifid. In a number of flowers there were two lobes alternating with the perianth segments, in others oblique or opposite the perianth segments; so that even when a stigma is bifid the orientation of the lobes is not constant. On the other hand, we have found no exception to the rule that the two embryo-sacs lie in the plane of the perianth segments. Johnson's figures (5, Pl. X, Figs. 1 and 6) show the embryo-sacs opposite the perianth segments in *A. oxycedri*, but he says nothing of stigma lobes. Heinricher (4) describes the stigma in this species as having an irregular rim above a funnel-shaped opening.

¹ The walls are thin, not as Johnson described for *A. oxycedri* 'thick, highly refractive, pitted'.

The flower as a whole has by June (Fig. 5) enlarged to twice its length the previous August, chiefly by intercalary growth in the region of the ovary. The nipple has enlarged and become conical, tapering at the tip. Its boundaries are not easily distinguished in sections, partly owing to the loose character of the endocarp tissue immediately surrounding it, partly perhaps to the fact that the material had been sent fresh for other purposes, and had not travelled well. The nipple is, however, still free, for after soaking in dilute potash it can be dissected out.

The embryo-sacs have enlarged, usually one more than the other. Our material does not allow the cytological details to be made out. It is, however, possible, in some preparations, to see that one or both embryo-sacs contain several nuclei. In view of Johnson's description of *A. oxycedri* (5) it is probable that about this time the embryo-sac develops an egg apparatus with a normal complement of nuclei; but whether before or after pollination has still to be determined.

Development of Fruit.

By the beginning of July the distinctive changes have already begun which lead up to the characteristic fruit. It is here that we are able to supplement previous accounts very materially. The structure of the mature fruit is so similar in *A. oxycedri* that the course of development is probably the same, and perhaps characteristic of the genus as a whole.

Embryo-sac. Beginning of July.

In our material collected by Professor Faull on July 4, 1925, the developing embryo-sac has thrust a haustorial extension down into the base of the nipple (Figs. 6 and 8). The main part of it, too, has enlarged and become more or less spherical, and in the most advanced cases is bulging laterally from the placenta.

The fertilized egg can readily be distinguished behind the outward growing end, separated from the embryo-sac wall by a single layer of endosperm cells (Fig. 8). It has already divided into two or four cells. The orientation of the first division wall does not appear to bear any definite relation to the axis of polarity of the embryo-sac; but the direction of this is not easy to gauge at all accurately. The next division walls are about perpendicular to the first, but may be oblique to each other.

After comparing these July pro-embryos with the smallest embryos found in our August material we conclude tentatively that no suspensor is formed, so that *Arceuthobium* is very like *Viscum* in the development of its embryo (9).

In July the endosperm is sometimes merely a single layer of peripheral cells that extends from the embryo end of the sac down towards, but not as far as, or into, the haustorial prolongation at the base (Fig. 8).

In other cases it completely surrounds the embryo and fills the greater part of the enlarged upper cavity. Even in August the endosperm has not encroached on the haustorium, which is then apparently empty. In July

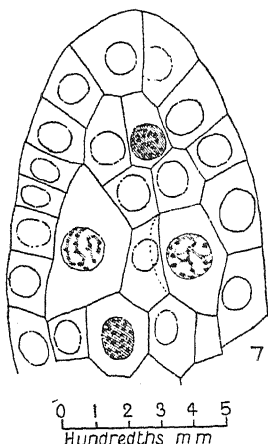


FIG. 7. Nipple from Fig. 4 enlarged, showing the two embryo-sacs (?). The nuclei of two other cells are shaded to indicate the contrast of structure between the embryo-sac nuclei and others.

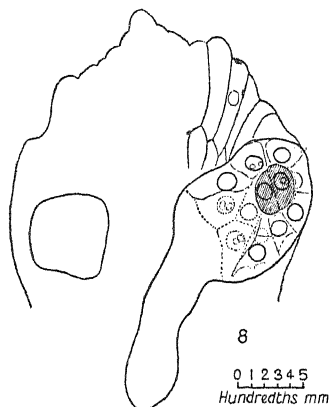


FIG. 8. Nipple, July 4 (cf. Fig. 6), showing enlarging embryo-sac, with haustorium, peripheral endosperm in upper part and two-celled pro-embryo (shaded); the second abortive embryo-sac is indicated.

material occasional degenerate nuclei have been found in the haustorium, which may represent the remains of antipodal cells, and sometimes endosperm cells extend into the upper part of it.

Endosperm and Embryo in August.

By the middle of August the endosperm has grown to a mass of tissue much larger than the nipple. The latter has been pushed aside and crushed, but can still be dissected out and shown to be, though flattened, otherwise of the same size and shape as before. The embryo has usually been carried upwards till it occupies an axial position in the ovary. Possibly rectipetality and symmetry of growth in the embryo-sac would account for this. In one case only, the embryo occupied a different position, half-way down, to one side, with its axis transverse.¹ Size of endosperm, size of embryo, and the proportion between the two vary rather widely.

Both Johnson (5, Pl. X, Fig. 10) and Heinricher (3; see Taf. II, Fig. 5) describe a cap of crushed tissue above the endosperm in *A. oxycedri*. Johnson interpreted it as part of the nipple ('ovarian papilla'). He says: 'In the ripe fruit the apical part of the papilla forms a sort of calyptra to the radicle and the basal part is thrust to one side by the enlarging endo-

¹ According to Treub (13) the embryo normally takes up a lateral position in *Viscum articulatum* (see his Plate II, Figs. 10 and 11).

sperm beneath the base of which it is visible as a yellowish patch of completely crushed cells . . . ' (pp. 149–50). In *A. pusillum* the whole nipple is pushed aside, and is found intact below the endosperm in the August fruit. It cannot therefore provide a cap in this species.

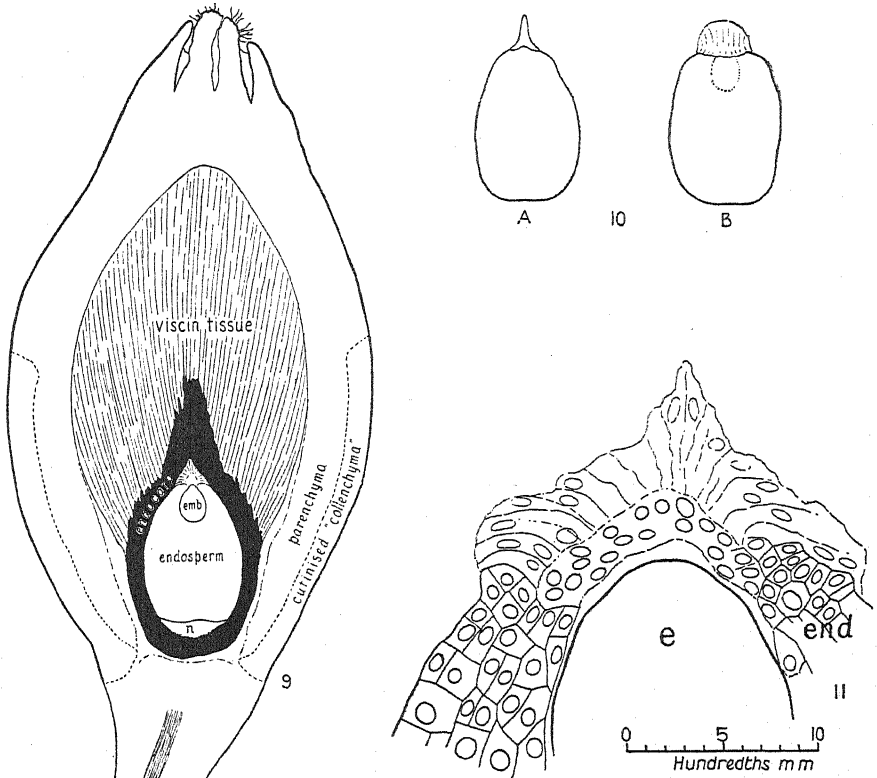


FIG. 9. Fruit, August 19, longitudinal section in plane between the perianth segments: *emb*, embryo, above which is the endosperm 'crest'; *n*, nipple (see text). Stigmatic hairs are still present. Round the lower part of the endocarp is a zone of small-celled tissue, with smaller masses of reserve material, which probably provides later for the separation of the parts that are expelled at dehiscence. $\times 39$.

FIG. 10. Endosperm dissected out whole from an August fruit, showing the 'crest'. A, as seen from a perianth segment; B, looking between the perianth segments (the dotted line shows the position of the embryo). \times about 25.

FIG. 11. Longitudinal section (slightly oblique) through the apex of the endosperm, *end*, and embryo, *e*, showing the 'crest'. The cells above the tip of the embryo have dense protoplasm, and their walls were too thin to be distinguished clearly.

Our fruits are probably not yet ripe, and provide an earlier stage of development, for their structure suggests a possible explanation of the cap. The cells of a limited area at the apex of the endosperm have elongated greatly, and together form a kind of crest or cock's comb with its breadth in the plane of the perianth segments (Figs. 9, 10, 11). Immediately underlying it is a zone of small cells rich in protoplasm, overlying the tip of the embryo. The function of the outgrowth may perhaps be haustorial at this

stage. It tends to 'flow over' the surrounding surface, but its limits are nevertheless fairly well defined. The adjacent superficial cells, closely fitting and more or less cubical, constitute a well-marked layer, which in some cases shows indications of cutinization. According to Heinricher (3) the outer wall of the endosperm in *A. oxycedri* is strongly cutinized. If the fully mature fruit of *A. pusillum* is similar in detail to that of *A. oxycedri* the embryo must elongate through the gap in the cutinized layer, at the apex of the endosperm, crush the crest and detach it from the rest of the endosperm as the 'calyptra'-like cap described by Johnson and Heinricher.

In the solitary case of a lateral embryo, no crest was found, either apical or lateral.

Even in the largest embryos seen by us no cotyledons could be recognized.

Endocarp and Viscin Tissue.

At the beginning of July the formation of the 'viscin' tissue has begun. The change is initiated in a ring-shaped zone of cells about half-way up the young fruit (Fig. 6). Thence it spreads downwards towards the level of the nipple and also across the already obliterated stylar canal. In the cells of this zone the characteristic deeply staining gelatinous masses break up and gradually disappear, the cells elongate in directions obliquely upwards and outwards and become spindle-shaped and tapering, while their walls become mucilaginous.

By the middle of August (Fig. 9) these cells have become very long and hair-like, the walls thick, the lumen almost obliterated in parts, and the contents reduced to slender irregular strings of granular matter in which the nucleus is still to be seen at some point.

The zone is originally several cells in thickness. Ultimately, elongation brings all the cells to lie parallel and more or less of equal length; but some taper to a point near the outer margin of the viscin tissue, where they may occasionally branch, others end in flattened expansions, so forming a more or less coherent boundary layer at the outer limit. At the base, many of them end in cell-like expansion, suggesting that only their upper part has undergone the change. Heinricher figures similar elements in *A. oxycedri* (3, Taf. IV, Figs. 2, 3, 7). These expanded bases are similar in all respects to the cells of which the rest of the endocarp, immediately surrounding the endosperm, is constituted. They are uniformly filled, each with a mass, brown in preserved material, of a very resistant substance of tough gelatinous consistency, with a marked affinity for safranin. A very similar material (there are variations in tint and clarity) is conspicuous in the cells of the parenchymatous tissues, where it is accompanied by starch grains. Similar material is also found in the outer cells of the endophytic strands (11, p. 410) and also in certain cells of the host. The fact

that these masses break up and disappear in the development of the viscin tissue shows that the substance is a reserve product—at least, notwithstanding its resistant character, it is mobilizable by the plant. Skrobischewsky, according to Pierce (8, pp. 101–2), found tannin present in the endocarp cells of *A. oxycedri*. In *A. pusillum* they are not sclerotic, as stated by Pierce for *A. occidentale*. The substance in question blackens with osmic acid. It also takes up Sudan III from a glycerine-alcohol solution: its original brown colour obscures this fact until the section is placed in sulphuric acid when the Sudan III turns a deep rich red. It also darkens in varying degrees with ferric chloride, in some cells turning merely slightly darker and in others, especially among the endocarp cells black, though the black colour is mostly given by cells which have no gelatinous masses, the reaction occurring in the peripheral substance. F. E. Lloyd (6, 7) has described masses very similar in their resistance to reagents, and in their tough gelatinous consistency, which he regards as tanned carbohydrate gels, related to mucilages. Heinricher applied numerous tests to the corresponding masses in *A. oxycedri* and remarks on their extraordinarily resistant nature (3, p. 12). Like Lloyd's tanned mucilage (which dissolved in time in Schultz's macerating fluid), they yielded only to oxidizing agents, slowly dissolving in Eau de Javelle and in aqueous chromic acid. In *A. pusillum* they are at least equally resistant. They do not dissolve in 40 per cent. formaldehyde as Lloyd's tannin masses did. They resist Schultz's macerating fluid in the cold for at least several days, though their colour pales very quickly to a lemon yellow; but they disappear on boiling.

‘Collenchymatous’ Sheath.

In July the upper part of the fruit, including the perianth segments and style, is fully mature. The viscin tissue begins to form just below, while the lower part of the fruit is still meristematic.

By August a very considerable intercalary enlargement has taken place. Just within the epidermis in this region a cup-shaped zone of cells has been differentiated, reaching to the base of the fruit, the walls of which are conspicuously thickened in a collenchymatous manner (Fig. 9). These walls do not, however, give the reactions of cellulose, but like cork or cuticle resist concentrated sulphuric acid, stain yellow with iodine, and strongly absorb Sudan III, cyanin, and alkannin. Heinricher describes the corresponding tissue in *A. oxycedri* as ‘suberized collenchyma’ (*das verkorte Collenchym*) (3, p. 8). He states that the walls swell and glisten like ordinary collenchyma when treated with 50 per cent. aqueous potash in the cold, and after boiling lose their glistening appearance, are poorer in substance, and give no cork reactions; the limiting layer next the lumen alone gives the cellulose reaction with chlor-zinc-iodine.

Evidently this tissue, to which an important role is attributed in the expulsion of the 'seed' (3), is another unique feature of *Arceuthobium*. We know of no other case on record of a suberized or cutinized collenchyma. It appears to offer an exceptional opportunity for the study of cutinization, and is under investigation from this point of view. The results up to the present appear to indicate that between limiting cellulose layers, next the lumina of the cells, is a gelatinous or mucilaginous filling, largely pectic, but possibly with some cellulose in its composition, heavily impregnated with lipid substances.

In our material the fatty substances are more readily removed than Heinricher found, but this may be a sign that the tissues are not yet fully mature. The cutinized layers of the epidermis in the region of the collenchyma (though not in the exposed parts above) are equally susceptible. Even 2 per cent. potash in the cold removes much of the cutinizing material, and after four hours or less in 10 per cent. aqueous potash, no stain is obtained with Sudan III. Boiling in 10 per cent. potash entirely dissolves the filling, leaving only the cellulose lamellae next the cell cavities. Alcoholic potash rapidly dissolves the 'cutin' and the walls collapse. Subsequent acidification results in drastic shrinkage, but the remains take up ruthenium red.

Nowhere in the tissues do the walls readily give a cellulose reaction with chlor-zinc-iodine, but after alcoholic potash an excellent reaction is given by the walls of the parenchyma, definite reactions also by the walls of the endocarp cells, and the inner layer of the wall in the viscin tissue, endodermis, and collenchyma. Thus impregnation of the walls with fatty substances is general. Fat droplets are very generally present—in the parenchyma cells among the starch grains at the periphery, outside the gelatinous masses; in the viscin cells scattered plentifully along the attenuated lumen, and very conspicuously as large drops in the peripheral limiting layer of the viscin tissue; also in the endosperm.

Notwithstanding the general impregnation of the walls with fatty substance, it is only the cuticle and the cutinized filling of the collenchyma which withstand the action of 70 per cent sulphuric acid.

It is curious, in view of the similarity in the reactions of these materials, that there is no continuity between the cuticle and the cutinized filling below the epidermis, so that after treatment with sulphuric acid the cuticle separates as a distinct layer.

SUMMARY.

In the anther the archesporium forms a continuous ring round a central 'columella', as in *A. oxycedri*. This is surrounded by a tapetal layer, an inner wall layer of shallow cells, and an epidermis which probably functions as an exothecium in the ripe anther.

The female flower bud in mid-August shows rising from the base of the ovary a dome-shaped 'nipple' (? placenta) in which two cells, probably embryo-sacs, are conspicuous. By early June, pollination has occurred and the embryo-sacs are usually pluri-nucleate.

By the beginning of July the fertilized embryo-sacs have put a haustorial extension down into the base of the nipple, have enlarged above, and are growing out sideways from the nipple. The one- to four-celled spherical pro-embryo is near the upper end. A single peripheral layer of endosperm cells extends downwards towards the basal haustorium. The characteristic 'viscin tissue', of elongated cells with mucilaginous walls and attenuated lumina, is just beginning to form in a ring-shaped zone below the base of the style.

In August the endosperm is a mass of tissue filling the enlarged cavity of the ovary. It has carried the embryo up to a median position in the fruit. The nipple has been pushed aside and crushed. At the top of the endosperm the peripheral cells have grown out to form a sort of crest which may be haustorial in function.

The embryo at this stage is usually an ovoid mass of tissue with its longer axis placed longitudinally; cotyledons are not distinguishable. No suspensor has been found.

In the so-called 'collenchymatous' layer of the fruit wall the thickenings consist of limiting layers of cellulose next the cell lumina, with a filling between them, similar in composition to the cutinized zone of the outer epidermal wall.

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Variations in *Botrytis cinerea*, Pers., Induced by the Action of High Temperatures.

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With Plate XXXVII and five Figures in the Text.

THE production of variants of *Eurotium herbariorum* from conidia which had been exposed to heat (Barnes (2)) led to experiments with *Botrytis cinerea*. These experiments yielded forms clearly different from the stock strain of *Botrytis*, and the work was continued in order to determine the degree of constancy possessed by these variants.

The strain of *Botrytis* used was obtained in December, 1926, from a strong growth on dead stems of *Helianthus annuus*; cultures were prepared from single conidia, and one of these pure cultures formed the starting-point of the work to be described.

CULTURAL DETAILS.

The original isolations were made on potato agar; the fungus grew freely on this medium, and formed conidia in abundance, but the crops of sclerotia were poor. Several synthetic media were tried, and of these Czapek's medium,¹ and a modified form of Reidemeister's medium² (Reidemeister (13)) were found to give good growth and moderate crops of sclerotia; the second medium has been chiefly used in this investigation.

All the synthetic media were prepared from the same lot of chemicals. Material was sterilized for twenty minutes at thirty pounds pressure; the depth of the medium was maintained at 3-4 mm.

With the exception of those cultures which were incubated for a special reason (p. 829) the cultures have been grown in the open laboratory,

¹ Sodium nitrate, 2.0 gm.: dipotassium phosphate, 1.0 gm.: magnesium sulphate, 0.5 gm.: potassium chloride, 0.5 gm.: ferrous sulphate, 0.01 gm.: cane sugar, 30.0 gm.: agar, 20.0 gm.: distilled water, 1 litre.

² Potassium nitrate, 5.0 gm.: magnesium sulphate, 2.0 gm.: dipotassium phosphate, 2.0 gm.: glucose, 50.0 gm.: agar, 20.0 gm.: distilled water, 1 litre.

and so subject to changes of temperature. During the colder part of the year the temperature ranges between 14–17° C.; in the warmer months it varies with the weather. Cultures were seldom exposed to direct sunlight, but they received much illumination from electric light, amounting probably to ten to twelve hours per day for most of the year. Owing to limitations of space it has been necessary to keep the dishes in stacks of five or six; as far as possible, any effects of uneven illumination due to stacking have been neutralized by frequent rearrangements. It does not seem likely that any given dish has been exposed to conditions differing essentially from those affecting all the cultures in existence at the time.

THE BEHAVIOUR OF THE NORMAL STRAIN IN CULTURE.

(a) *On Synthetic Media in the Laboratory.*

When cultures are started from conidia, whether transferred by a cool wire or in drops of sterile water, growth is usually just visible to the naked eye in twenty-four hours, and an agar-surface 9 cm. in diameter is covered in about five days. During the first days of growth the mycelium spreads evenly (Pl. XXXVII, Fig. 10) over the medium, penetrates the agar but slightly, and appears as a low, even turf of white and sterile mycelium, 2–3 mm. in height. The aerial mycelium is loose and freely branched; it usually shows but vague indications of radiating main hyphae. By the fourth or fifth day the central region is occupied by a rather dense cushion, about 5 mm. high and 3–5 cm. in diameter, falling steeply to the prostrate margin of hyphae advancing over the medium; immature conidiophores may now be seen on the central cushion. When the margin of the agar is reached appressoria form freely against the glass, and a peripheral ring of aerial mycelium with conidiophores develops rapidly. At this stage conidiophores are forming centrifugally on the central cushion, centripetally on the marginal ring. There was no evidence that the first conidia to mature fall, germinate, and contribute to the development of the colony (Brierley, 6, p. 140); by the time that mature conidia are present it appears that the surface of the agar is too well covered to allow of the establishment of younger colonies.

As the two areas on which conidia are forming approach one another sporulation decreases, so that a somewhat thin crop of conidiophores is formed in a zone lying a centimetre or so from the periphery of the agar; rarely such a zone may be left almost bare of conidiophores.

The conidiophores branch freely. Owing to the dense growth in cultures of the stock strain it is impossible to follow the branching, but in the thinner growths of some of the variants, and of the stock strain on poor media, it can be seen that the first fertile axis bears a terminal head

of spores, with a whorl of branches arising immediately below it; each branch repeats the development, and so on. In this way the dense turf of conidiophores is built up.

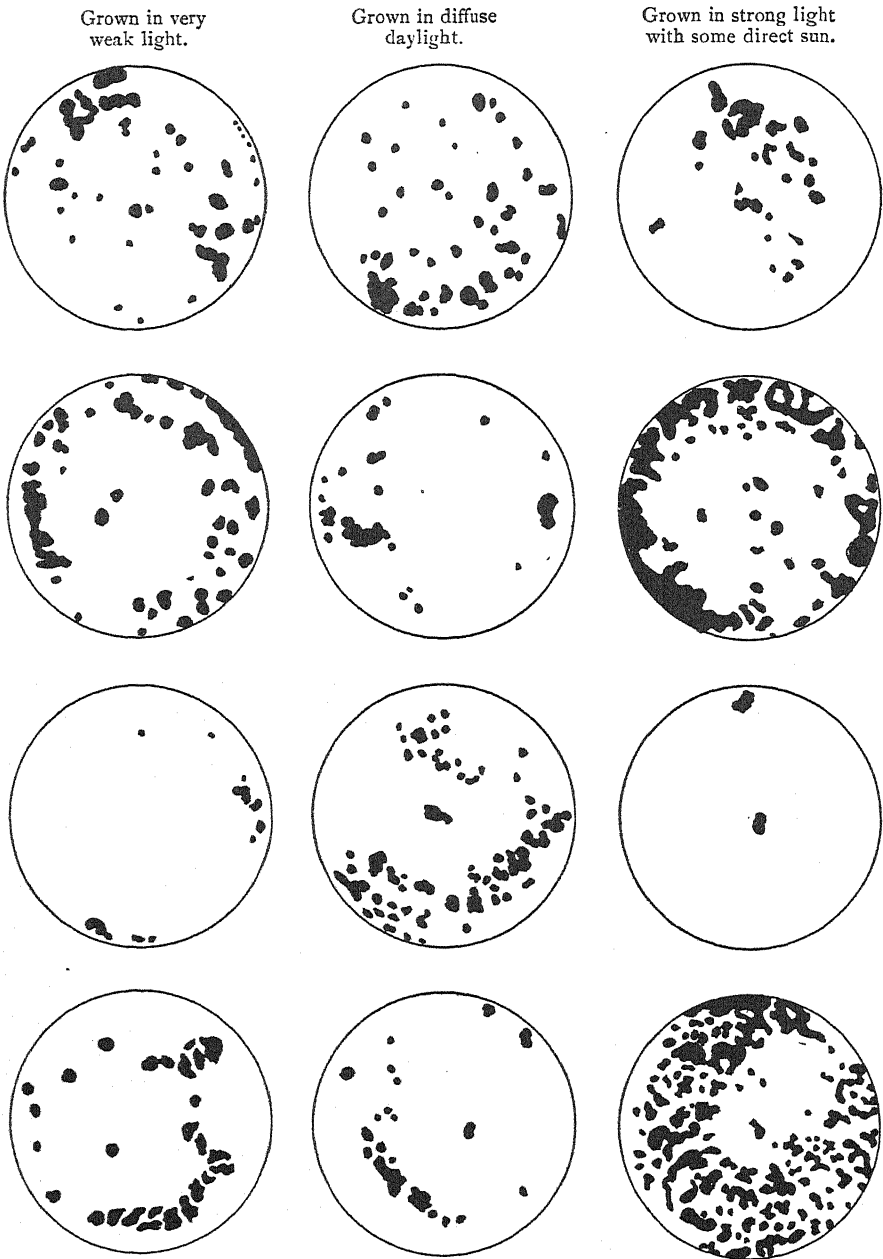
A culture ten days old, viewed from above (Pl. XXXVII, Fig. 1), appears to consist of an even turf of brown conidiophores, but the presence of the thin zone is revealed when the culture is held against the light. Cultures of this age show the first signs of sclerotia as small, whitish hyphal aggregations lying on the medium where conidia are few. The rudiments soon enlarge, remain white for a day or two, then become yellow and translucent, and finally black and opaque: the edge darkens first, and the larger the sclerotium the slower the darkening of the centre. After the first rudiments have appeared, others form nearer the periphery, some against the glass; finally, a few belated sclerotia may arise in the central region. Rarely, these fail to mature, and remain as thin brownish aggregations of indefinite outline; if they blacken they may do so more slowly than those already formed. In cultures three weeks to a month old the sclerotia are usually all black and opaque, and no more develop.

Individual sclerotia attain an average diameter of 3 mm. They are flat, a millimetre or so in thickness, or, if convex upwards, present an illusory appearance of thickness, for the convexity is due to the upward curvature of the sclerotium, with some inclusion of the medium in the lower face. The crusts formed by the fusion of several rudiments usually remain flat, and seldom exceed 1 cm. in any horizontal dimension.

The richness of the crop of sclerotia may be influenced by the manner in which the culture is started. In single spore cultures, or in cultures started from a few conidia placed in the centre of the dish, or from two or three conidia well spaced on the surface of the medium, a crop of seventy to over a hundred sclerotia may be obtained; from dense sowings sclerotia are seldom obtained. However, cultures have been observed in which sclerotia have been scantily produced, or have failed to appear, even when all conditions would seem to have favoured their development. The tracings given in Text-fig. 1 show how erratic the production of sclerotia may be in cultures prepared in the same way and subjected to the same treatment.

When cultures are started from sclerotia they develop much like those from conidia. The production of aerial mycelium and of conidiophores is, however, greatly reduced, and the crop of sclerotia is increased, several hundred often being obtained; conidial transfers from such cultures show the ordinary behaviour.

As cultures age further developments are noted. When there are few or no sclerotia, and the culture is about a month old, tufts of aerial mycelium arise among the old conidiophores, from hyphae on and in the medium.



TEXT-FIG. 1. Twelve cultures of the normal form of *Botrytis cinerea* grown on Reide-meister's medium. All were inoculated with a few conidia placed in the middle of the agar. The erratic production of sclerotia is clearly shown. Camera lucida tracings. $\times \frac{7}{18}$

These tufts may be loose, and greyish or whitish ; less often they are dense, and then distinctly tinged with pink, or, if developed in strong light, greenish. The looser tufts form large, pitchy black appressoria against the under side of the lid ; the denser tufts usually form smaller appressoria, which may remain brown and translucent. Very few conidiophores are formed on these tufts.

When sclerotia are present, and are five to six weeks old, they give rise to dense groups of conidiophores, which do not branch freely ; after the conidia are ripe, tufts of whitish, and usually sterile mycelium, arise from and around the sclerotia.

The formation of such tufts of aerial mycelium is a general phenomenon in old cultures. As a rule they remain localized, and attain a diameter of 1-2 cm., but they may increase so much as to occupy completely all the free space in the dish, hiding the older growth ; ultimately all collapses to a thin, dirty brown, papery sheet lying on the medium. The production of the tufts is usually accompanied by the secretion into the medium of a brownish-purple or blackish stain, lying below the points of origin of the renewed growths. Cultures inoculated with pieces of mycelium, or with conidia, taken from the tufts, do not show any abnormality.

Appressoria are formed freely by the stock strain. They appear as soon as the spreading mycelium touches the side of the dish, and develop readily against pieces of glass strewn on the surface of the agar. At first white and translucent, they soon turn brown, and then blacken ; they may attain a diameter of over 1 mm. and simulate small sclerotia. Small appressoria are also formed in large numbers by the peripheral ring of aerial mycelium, and lie in a crowded thin circular line where the lid rests on the side of the dish.

The foregoing account is based on the observation of the stock and control cultures which have been made during the investigation ; of these cultures, 239 in number, none has shown any marked divergence in its characters. Observation of these cultures has not suggested that the stock strain has shown any indication to vary during nearly three years of culture in the open laboratory ; nothing has been seen in any of the cultures which could have been isolated and grown as a recognizable variant. Further, it has not been possible to show that transfer from one medium to another may lead to the development of any change in the behaviour of the fungus, other than an obvious and temporary reaction to the medium.

(b) Incubated Cultures of the Stock Strain.

Systematic work on the effect of incubation at various temperatures on the stock strain was not undertaken ; from time to time, however, some

cultures were grown in incubators, in order to shed light on some of the peculiarities of the variants.

At temperatures between 25° C. and 27° C., on Reidemeister's medium, the general rate of growth did not appear to be essentially different from that observed in control cultures in the laboratory at about 20° C., but the production of conidia was less, and that of sclerotia slightly increased. In one culture, sclerotia formed after six days, and blackened normally. The production of sterile aerial mycelium was increased, and this, as well as the appressoria, often had a pinkish tinge. Greenish tufts also appeared, and in these, as well as on the surface mycelium, microconidia were found. In two cultures grown at 27° C. microconidia were so profusely developed on the surface of the agar that wide, dark green areas appeared, simulating infection by *Penicillium*. In incubated cultures microconidia were obtained in five days; in cultures at laboratory temperatures they have rarely been seen, and then only after a month or so of growth.

More interesting results were obtained at slightly higher temperatures. Growth did not occur at a temperature higher than 31° C. On Reidemeister's medium, at 30° C., growth was very poor. It usually stopped after incubation for eight days, when the colonies had attained an average diameter of 15 mm.; controls of the same age have a diameter of 90–92 mm. The growth was low, completely sterile, and consisted of a mass of irregular swollen, darkly-stained hyphae, with vacuolate contents, covered by a short turf of thin whitish filaments. Beneath the centre of the colony the medium was stained black, and in general appearance the cultures strongly resembled cultures of *Cladosporium*. Some of these cultures were left in the incubator for a further seventeen days, without any change in size or aspect. Transfers of mycelium failed to produce growth, either in incubated cultures, or at ordinary temperatures. Yet, when the cultures were brought back to laboratory temperatures, new growth soon appeared, and in a few days a perfectly ordinary growth, with conidiophores and sclerotia, surrounded the dense colony, above which growth did not appear.

Although sporulation was diminished or suppressed by growing the stock strain at rather high temperatures, and although definite changes in the manner of growth were obtained in this way, transfers of conidia formed in these cultures, either in the incubator or after return to a lower temperature, always gave normal cultures at laboratory temperature.

Efforts were also made to produce the white sterile form described by Beauverie (3, 4) and by Beauverie and Guilliermond (5). Wet earth in Petri dishes was inoculated with conidia of *Botrytis*, and repeated transfers were made of mycelium obtained in the incubator and of conidia obtained after the cultures had been placed in the laboratory. Growth was always very weak, but the white sterile form did not appear. Similar attempts

were made with Petri dishes incubated inside a closed vessel containing water, to maintain a humid atmosphere, and with stoppered bottles containing wet earth. The white form did not develop, and some conidia always appeared when the cultures were allowed to stand at laboratory temperatures. Cultures started from these conidia showed nothing abnormal. Brierley (6) records that he could not obtain the sterile form.

These experiments with incubated cultures were not carried far, but enough was done to show that the strain of *Botrytis* used is extremely sensitive to rise of temperature, and shows a rapid falling off in the rate of growth even when the rise is slight. The production in incubated cultures of much sterile aerial mycelium at the expense of sporulation, the early development of microconidia, the appearance of a pink colour in the aerial mycelium and appressoria, and, at higher temperatures, slackening of the rate of growth and staining of the medium, are all characters which have been found in one or other of the variants presently to be described.

(c) *The Effect of Hot Weather on the Stock Strain.*

The hot weather in July, August, and September, 1929, furnished indications supporting the observations made on incubated cultures. During this period the laboratory temperature remained for days not lower than 20°C., and sometimes rose above 30°C. The cultures of the normal form developed much aerial mycelium, showed great reduction in the crops of conidia, formed many pink appressoria, frequently stained the medium, and yielded few sclerotia. In subsequent cooler weather these abnormalities disappeared, and the strain resumed its ordinary behaviour.

EXPERIMENTS WITH HEATED SPORES.

During 1927, after it had become apparent that the stock strain was reasonably constant in character, a few attempts were made to obtain growth from heated spores; the methods used were those employed at the time in the work on *Eurotium*. It was found that the conidia of *Botrytis* were more readily killed by heat than were those of *Eurotium*, that there was delay in the germination of some of the surviving spores, that a tendency to the production of an enhanced crop of aerial mycelium was apparent, and that some of the sclerotia darkened more slowly than normal ones, requiring three weeks to a month for the completion of the process. The aerial mycelium was usually sterile, but occasionally bore a few weak conidiophores which seldom yielded good conidia. It was often greenish in tinge, but though microconidia were suspected they were seldom seen; most of the green colour was due to pigmentation of the hyphal walls.

In 1928, when the experimental work on *Eurotium* was completed, further attempts were made to obtain changes in *Botrytis*. For most of

the trials the following method was used. Weak suspensions of conidia were made in distilled water and shaken until it appeared that the spores were well distributed in the liquid. Quantities of 0.5 cc., were withdrawn by means of a narrow tube, and conveyed separately to the bottoms of test tubes, care being taken to avoid the deposit of liquid on the sides of the tube. The charged tubes were immersed as deeply as possible in a water bath at a known temperature, and held there for two minutes, with persistent shaking, so that the small volume of suspension was thrown in a thin layer against the hot sides of the tube. After withdrawal from the bath, the tube was cooled under a bell-jar; later, drops of suspension were withdrawn on a glass rod, and conveyed to plates of agar. Control cultures were inoculated from the unheated suspension. Suspensions of constant density could not be prepared, but in most of the suspensions used, an average drop, such as was used for inoculating a plate, contained about thirty spores.

All water and apparatus were sterilized before use, and all necessary precautions were taken to avoid contamination. It was found convenient to use test-tubes closed by means of a specimen tube of slightly greater diameter inverted over the mouth; this arrangement formed a sort of deep and narrow Petri dish, and manipulation was easier than when a plugged tube was employed.

In all, 398 attempts were made in this way, including 42 controls. The exposures to heat were made over the range 48–80° C., at intervals of 2° C. The controls all gave normal cultures. Of the 356 dishes inoculated with heated spores only five gave any growth. Four had received drops of suspension which had been heated to 54° C.; the suspension in the fifth had been heated to 66° C. Of the first four, one culture was apparently quite normal, two were distinguished by the production of much aerial mycelium, and one yielded colonies of rather loose growth; these four cultures were not further investigated. The fifth culture ultimately gave some light coloured sclerotia; it was the starting-point of the subsequent work, and is described as culture A, on p. 834.

The absence of contamination from any of the dishes of this long series is an indication of the trustworthiness of the methods used.

A few cultures were started from singed spores; a mass of hyphae and conidia was taken on a hooked wire, well singed in a flame, and transferred to a dish. Growth seldom arose from the charred mass, but conidia which had fallen clear of it during transfer frequently germinated. Colonies from these generally produced a tuft of sterile aerial mycelium when they encountered the inoculum, suggesting the presence in it of a substance capable of affecting the growth of the fungus and causing sterility. As viable spores could be shaken out of the transferred material, it seems likely that similar spores remained inside it. Their usual failure to germi-

nate was probably due to the presence of deleterious substances in the charred mass. The more striking abnormalities observed in these cultures occurred in mycelia starting clear of the inoculum, and sometimes not reaching it; the changes noted may therefore be reasonably ascribed to the direct effect of heating upon the spores.

A dense, black stained growth appeared as a single colony in two cultures inoculated with singed spores; in a third it was accompanied by one normal colony. In all three growth arose from spores which had fallen clear of the charred material. The mycelium consisted of a mass of blackish hyphae with barrel-shaped segments, lying on and in the medium, covered by a scanty tuft of unhealthy-looking white hyphae. Although attempts at the formation of conidiophores were seen, conidia could not be found; efforts to obtain subcultures from the colonies, by transfer of mycelium, were fruitless. In its general characters this form resembled the stunted growths obtained when the ordinary form is grown on Reidemeister's medium at 30° C., but, unlike those growths, it did not yield conidiophores at laboratory temperatures.

A low, densely branching colony, also grown from a singed spore, appeared to be identical with the dense white form which will be described in Series I, VI, and VII; it was the only colony to develop in the dish.

Many cultures derived from heated spores were distinguished by the production of an unusually heavy crop of aerial mycelium, with reduced formation of conidiophores and occasional development of faintly green tufts of hyphae. All these phenomena were encountered in cultures of the normal form, grown in the incubator; some were also observed in old control cultures. Their appearance in young cultures, inoculated with heated spores, indicates a slight disturbance of the normal course of development. Efforts were not made to perpetuate these slight changes, nor were attempts made to obtain subcultures from the slightly modified forms numbered 7 and 8 in Table I. All were probably temporary modifications, and would not have repaid investigation.

As in the work on *Eurotium*, so with *Botrytis*, the most striking variants were observed in cultures in which few or only one spore germinated; when a number of spores germinated after exposure to heat, the cultures gave either normal growth, or slightly altered forms, often only recognizable as variants in early stages of growth.

The experiments with heated spores were discontinued as soon as a fair number of positive results had been recorded. The investigation was regarded as an extension of the investigation of *Eurotium*, and it was not considered necessary to accumulate a large number of abnormal forms.

TABLE I.

Summary of Experiments with Heated Spores.

	Method of Heating.				
	Hot Lead.	Hot Wire.	Singed Spores.	Hot. Water.	Totals.
Cultures from heated spores :					
1. No growth	60	2	11	351	424
2. Dense, black stained growth	—	—	3	—	3
3. Low growth with dense branching	—	—	1	—	1
4. Slowly darkening sclerotia	2	1	4	1	8
5. Much aerial mycelium, often with a green tinge, and with greenish ap- pressoria	3	12	19	2	36
6. Greenish aerial mycelium with de- layed formation of conidia	—	—	12	—	12
7. Weak growth in young stages	6	7	1	—	14
8. Loosely growing colonies	—	1	—	1	2
9. Normal growth	—	7	12	1	20
Totals	71	30	63	356	520
Controls, all with normal growth	23	8	24	42	97

The more important abnormalities are included under items 2-4 of Table I. The large number of experiments in which all the spores were killed is noteworthy.

THE VARIANT FORMS.

I. THE ORIGINAL VARIANT CULTURES.

Culture A. In April 1928 a single colony appeared in a dish inoculated with a suspension of spores which had been heated to 66° C. for two minutes. Indications of growth were seen five days after inoculation, and it was clear that only one spore had germinated. Once started, the colony grew as fast as ordinary colonies, but at an age when a control culture contained an even growth of old conidiophores, and black opaque sclerotia, Culture A showed much whitish, sterile aerial mycelium and a few whitish sclerotia; the appressoria were pale yellow. When the culture was 43 days old it contained 21 black opaque sclerotia, all bearing tufts of conidiophores, and eight small, light coloured ones not bearing conidiophores. The culture was kept under observation for a further 62 days; five light coloured sclerotia were still present. They were removed separately, well rubbed with sterile glass needles in six changes of sterile water, and used to start fresh cultures. Three, each about 1 mm. in diameter, were transferred whole; two others, each about 2 mm. in diameter, were halved, and used to inoculate four more dishes. All the

sclerotia were discoid and soft; the two which were cut showed slight superficial darkening and a white medulla.

Growth was obtained in all seven, and in contrast to cultures started from normal sclerotia none of these seven yielded many sclerotia. Such of the seven as formed the starting-points of series of cultures will be described in the series to which they belong.

Culture B. In March 1928 a colony derived from spores heated in a capillary tube on molten lead produced two sclerotia which were still translucent in early July. These sclerotia were washed and transferred separately to fresh media. The resulting colonies produced few conidia, much brownish aerial mycelium, and stained the medium strongly. Few transfers were made from these cultures. A similar variant was afterwards obtained in the progeny of Culture A; it is described in Series II.

II. THE SERIES OF VARIANT FORMS.

Almost all of the cultures of the series now to be described were grown on Reidemeister's medium, and all were grown at laboratory temperatures. When a variant was first recognized, a single colony was isolated as a starting-point for further investigation, or, when conidia were slow to form, the end of a hypha was transferred. Conidia were generally used in making the routine transfers, and these were taken from cultures which showed most strongly the characters of the variant to be perpetuated. From time to time small portions of mycelium were transferred from regions showing the abnormalities characteristic of young cultures of the variants. As it was desired to find how long the variants could preserve their characters under the conditions of culture employed, few subcultures were made from cultures showing a definite approach to the normal form.

The numbering of the series does not denote the order in which they arose. They are described in the most convenient sequence.

Series I.

The original culture of Series I arose from half a sclerotium taken from Culture A. A heavy, white, and sterile cottony mycelium formed, and bore conidiophores freely by the tenth day. Rudiments of sclerotia appeared when the culture was 29 days old, but good sclerotia never developed. Transfers were made from this culture when it was 19 days old; these cultures resembled the parent in the production of much cottony aerial mycelium. In one, sclerotia never formed; in the other a few sclerotia appeared, and of these the larger blackened somewhat, but were still translucent after 89 days, while the smaller remained pinkish and light in colour. Conditions of this sort recurred in cultures made during August and September 1928, with indications of the formation of a central mass of indifferently formed sclerotia, recalling the phenomena described more fully in Series III. Not until November 1928 did it become clear that

the cultures of Series I were settling down to two distinct types; since that time these have persisted, though in many of the cultures, as will be shown below, the two have appeared together. In association with the rather weak tendency to produce pink sclerotia noted from time to time in this series, indications of poor growth, similar to the phenomena common in Series III, have been observed.

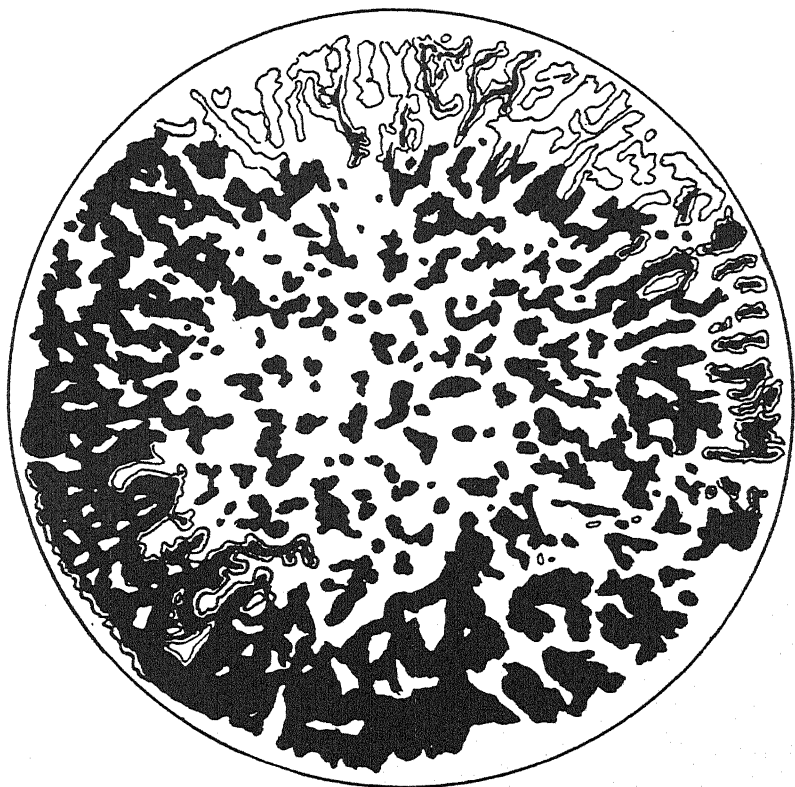
I a. *Cultures producing many sclerotia.* Growth is as fast as in control cultures, but when a culture is two to three days old it is readily distinguished from a control of the same age by the much thinner and lower growth, the formation of a dense sheet of superficial hyphae about the point of infection, and the presence of a relatively small number of weakly branched main hyphae straggling outwards across the medium from the dense centre. Young colonies produce little aerial mycelium, and show a straggling edge of separate main hyphae (Pl. XXXVII, Fig. 12), which contrasts strongly with the dense even margin of normal cultures (Pl. XXXVII, Fig. 10). The medium is rapidly acidified around the colony. When freshly prepared, the medium has a pH of about 7; around the edge of a growing colony, a pH value of 3 may soon be demonstrated. If the medium is prepared in such a way that it is slightly turbid with suspended magnesium phosphate, the effect of the acid secreted may be seen in the appearance around the fungus of a clear region a millimetre or so in width, due to the solution of the phosphate. This effect is not specially apparent in cultures of the normal form, which, however, acidifies the medium.

The denser central region extends as the colony grows, and by the time that the edge of the medium is reached a thin central cushion of aerial mycelium is present. A weak marginal ring soon forms, and on it and on the cushion scanty crops of conidiophores appear. This occurs when the culture is nine to ten days old, and at the same time rudiments of sclerotia begin to form in very large numbers, generally in radial streaks. Fusion occurs freely among the rudiments, so that large flat crusts of sclerotia form. This variant is the only one of those investigated which shows a definite reaction to Czapek's medium, for on that medium it forms specially good crops of sclerotia. On both the synthetic media used the crusts may cover 75 per cent. of the surface of the agar.

There is little constancy in the way in which the sclerotia darken. In many cultures darkening began by the appearance on the surface of the sclerotia of small, dark brown areas, which spread and coalesced; in many others, darkening began at the edges and spread inwards, as in the normal form; in still others the central parts of the crusts darkened completely, but the edges remained brown and translucent for weeks. Often in the earlier cultures a pink tinge was apparent in the young sclerotia. Usually these darkened completely, but occasionally some sclerotia remained very flat and pink. This usually occurred in vaguely defined sectors, and

conidia taken from above these areas sometimes failed to yield good cultures, while at other times they produced the form next to be described.

I b. *Colonies of low, dense white growth.* Growth is rather slower than in controls, but in early stages the colonies are very like those of I a.



TEXT-FIG. 2. A culture of Variant Ia with sectors of pink sclerotia (shown by plain outlines), black sclerotia with pinkish edges (shown by outlines and solid centres), and black sclerotia (solid black). Camera lucida tracing. $\times 1.15$.

The central area is, however, denser, and the straggling edge more obvious, as the hyphae produce large numbers of short lateral branches which arise close together and form conspicuous white groups. As a result of this dense branching the course of the main hyphae may sometimes be traced in cultures months old. Acid is secreted more freely than in I a, so that the acidification of the medium proceeds rapidly, and the solution of magnesium phosphate is well seen. Most of the forms of *Botrytis cinerea* which have been investigated give rise to a plectenchymatous layer on the surface of the medium. In I b this layer is developed strongly, and as the cultures age the plectenchyma thickens, is thrown into folds, and stains yellow below. Investigation of this layer showed the presence in it of a

considerable number of irregularly swollen hyphae tightly crowded together, and suggested that it might be regarded as a kind of imperfectly formed generalized sclerotium, an expression of the strong tendency to form sclerotia which is clearly present in I a. In cultures a month or more old small irregular patches of darkly stained regions are abundant beneath the plectenchyma.

The production of conidiophores is always scanty; they do not arise on a central cushion, nor on a marginal ring of aerial mycelium, but appear in scattered patches on the surface of the culture. It is seldom that well-formed conidiophores are seen; if such occur, they usually form at the edge of a colony which has ceased to extend before it had reached the edge of the dish. More often areas of the surface growth assume a biscuit colour, and conidia are formed on those hyphae in a somewhat casual manner. The conidia do not differ morphologically in any way from those of control cultures.

Sclerotia are rarely formed, and, when they do appear, they are limited in number, seldom exceed a millimetre in diameter, are polished, and densely black. These sclerotia may form around the point of infection, or, if a few conidiophores have been formed at the margin of the colony, they may lie beneath those structures. In one culture rudiments of sclerotia appeared in enormous numbers, but they failed to mature. The formation of conidia and sclerotia by I b has not been found to bear any relation to the age of the culture.

Conidia taken from conidiophores arising above areas bearing pink sclerotia in I a have on many occasions yielded mixed cultures of I a and I b. Mycelial transfers from I b in such cultures have yielded this form only. The examination of conidia has not revealed any differences which can be used to distinguish the conidia of the two variants. Thick plantings of conidia from I a give small, dense colonies resembling I b, but the resemblance is only apparent, for if these colonies have room to grow they give I a. The general results of the investigation of Series I suggest that I a is a somewhat instable form, able to produce pink sclerotia occasionally, and then yielding I b; I b, on the contrary, seems to be a permanent variant. A similar form will be referred to in the description of Series VI and VII.

When transfers of few conidia are made from a good culture of I a, and care is taken to avoid choice of material from areas bearing abnormal sclerotia, this variant can be perpetuated cleanly, and retains in a striking manner its power of forming large crops of sclerotia.

The resemblance of I b to *la toile*, described by Beauverie (3, 4) and by Beauverie and Guillermond, (5) is considerable, but, as it always forms some conidia, and may form sclerotia, it is distinct from that sterile form. Examination of the material has not revealed convincing evidence of the

presence of the modified conidiophores recorded by these authors in their *forme intermédiaire*, from which also I b seems to be distinct in its constant behaviour.

TABLE II.
Analysis of Series I, in Periods of Three Months.

Period.	Class of Culture.						
	1.	2.	I a.			I b.	
			3.	4.	5.	6.	7.
1928.							
July-September	—	10	2	2	—	—	—
October-December	2	1	1	2	15	9	11
1929.							
January-March	—	—	—	1	7	—	3
April-June	6	—	—	3	16	1	4
July-September	4	—	—	1	16	20	11
Totals	12	11	3	9	54	30	29
							148

- Notes.—1. Cultures showing poor sterile growth.
 2. Dense cottony cultures.
 3. Cultures of I a form, but with central sclerotial mass.
 4. Cultures of I a form, but with some pink sclerotia.
 5. Good cultures of I a.
 6. Good cultures of I b.
 7. Mixed cultures of I a and I b.

*Series II.*¹

Some of the earlier cultures of Series IV yielded a few poorly formed sclerotia which remained white. Seven of these were removed and transferred separately to fresh dishes. Six of the cultures agreed in all respects with cultures raised from normal black sclerotia. The seventh produced a colony distinguished by the formation of an exceptionally heavy crop of greyish to brownish aerial mycelium, with a scanty crop of conidia, and a few pinkish sclerotia. Conidial transfers from this culture formed the beginning of Series II.

Early growth is as rapid as in controls, but usually somewhat looser. When a culture is about nine days old a heavy tuft of nearly sterile aerial mycelium arises in the centre; it is at first greyish, but soon becomes a dirty brown, spreads in the free space in the dish, and often fills this so completely that the marginal ring of conidiophores is almost hidden. As the dense centre forms, a yellow stain appears in the medium below it, and by the time that the culture is a month old the medium is heavily stained; in colour the staining ranges from yellow, through brown, to purple and black. The staining is not uniform, but in round patches, which suggest the presence of sclerotia; these, however, have not been found in cultures

¹ See note at end of paper.

of Series II. The formation of appressoria is rather capricious; such as appear are normal. Cultures of this series have been under investigation since October 1928; owing to the great consistency shown, thirteen only have been made. The pronounced staining of the medium is the principal character of the cultures of Series II. Heavy staining has not been observed generally at ordinary temperatures in cultures of *Botrytis*. It may occur in small patches in old control cultures, under the tardily formed tufts of more or less sterile mycelium, and it frequently appears in old cultures of the dense white form of Series I, VI, and VII.

Series III.

The first culture was derived from one of the half sclerotia from Culture A. It started rather weakly, produced a scanty crop of conidia and a few small sclerotia of irregular outline, which took four months to darken. The sister culture was of similar character, and was not further used. Conidial transfers from the first culture gave subcultures which produced an abundance of white, sterile, aerial mycelium, very few conidia, and poor, thin, whitish sclerotia, which remained so for months. These characters were soon lost in succeeding transfers, and, as the investigation has proceeded, the cultures of this series have shown a general drift back to normality. The most general characteristic of the cultures of Series III has been apparent in early stages; the conidia have shown very consistently a weakness in starting, as compared with control cultures. This weakness has ranged from a total inability to germinate to a lag in starting amounting to about twenty-four hours. It has not, however, been possible to establish one definite variant as characteristic of the series, and the cultures fall into the following classes:

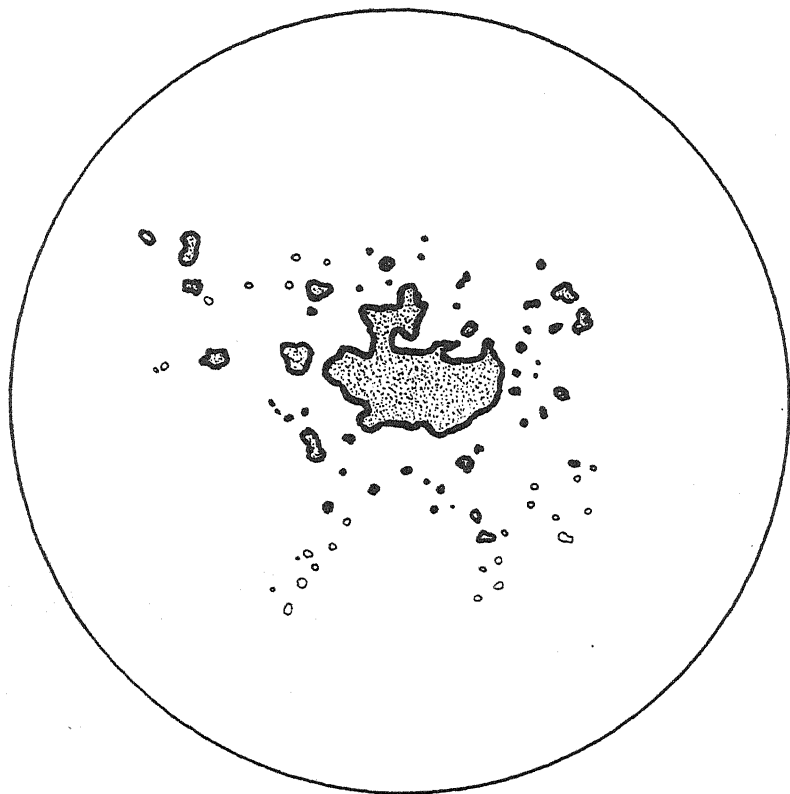
III a. Cultures of very weak growth. Of the 174 cultures which are shown in Table III, 41 (including 34 conidial transfers and 7 mycelial transfers), have failed to develop growth of macroscopic size, and 16 (including 12 conidial transfers and 4 mycelial transfers) have given weak and persistently sterile colonies; in all, microscopic examination showed that the inoculum had been successfully transferred; in many, ungerminated conidia, or conidia showing but faint signs of growth, lay for weeks on the surface of the medium. The inocula which gave these curious results had always been taken from above areas in the parent cultures which bore pink sclerotia, or from a portion of the mycelium which showed specially weak growth. Table III shows that the mere age of the inoculum has nothing to do with the failure to produce a strong colony, and it was often found that sister cultures, started from the same lot of conidia, and treated in exactly the same way, sometimes gave good growth, and sometimes poor or no growth.

After a connexion had become apparent between weak growth and

conidia taken from special areas in the parent cultures, many samples of conidia from such areas were examined, but significant differences in either appearance or size could not be found. In stained material it was seen that the conidia contained on an average about five nuclei, which were too small for satisfactory observation, and differences were not noted. Examination suggested that the hyphae of a weakly growing mycelium were more vacuolate than those of a more active mycelium, but nothing definite could be established. The fact appears to be that if conidia are taken from above pink sclerotia, or if mycelium be taken from regions of feeble growth, such material is likely to give rise to weak cultures; the explanation is still to be found.

III b. Cultures showing a central aggregate. The conidia take about a day longer to germinate than do conidia in control cultures, and in early stages of growth almost all the mycelium lies on or in the medium, and often shows extraordinary curvatures in the main hyphae (Pl. XXXVII, Fig. 11). Ten days may be required to cover a surface which is covered by the normal form in five to six days, and, in the low growth, radiating areas of somewhat greater density soon become apparent. When the culture is about a fortnight old it bears a scanty crop of conidiophores scattered about the surface and a thin central cushion of mostly sterile aerial mycelium. Rudiments of sclerotia appear crowded together, but at first separate from one another, under the central cushion, and others develop in the radiating areas of denser growth. These areas become narrower as they pass outwards, so that a vaguely stellate appearance is suggested, the points of the star reaching about half-way across the dish. The sclerotial rudiments in the centre assume a distinctly pinkish tinge as they develop, and soon fuse into a central aggregate; in this, the components are at first indicated by reddish lines of separation, but as the whole darkens the lines disappear. The larger sclerotia in the radiating areas enlarge, become pinkish, but usually remain separate; those at the ends of the arms enlarge but little, and are persistently pink; those nearer the central mass may ultimately blacken completely (Pl. XXXVII, Fig. 4). One of the early cultures of this sort, when seventy days old, showed a faintly brown, translucent central mass, with scattered brownish sclerotia around it, and small pink ones lying peripherally; the general appearance of the culture is shown in Text-fig. 3. As the work proceeded, this tendency to produce a central aggregate became much weaker; transfers sometimes gave a central aggregate, sometimes yielded cultures with pink sclerotia, but not a central aggregate, and sometimes produced cultures belonging to Class III d. A culture which was made five months after the one just described, by transfer of conidia from a parent showing a central aggregate surrounded by a fair number of black sclerotia, gave signs of the formation of a central aggregate when it was eighteen days old; the rudiments failed

to fuse completely, and many black sclerotia arose subsequently in the peripheral region. The appearance of this culture when seventy days old is shown in Text-fig. 4; there are but vague indications of any radial arrangement, and the old culture, had its history not been known, would

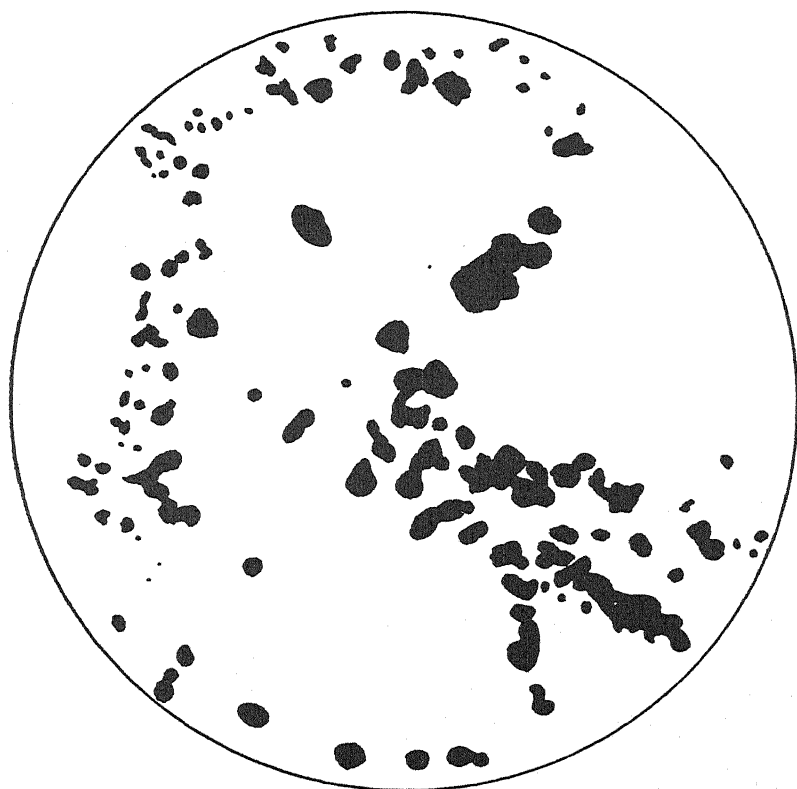


TEXT-FIG. 3. A culture of Variant III b grown in October 1928. The central aggregate is well defined, and the radiating groups are clearly shown. Sclerotia filled in by dots remained brown and translucent; sclerotia shown in outline remained pink. Camera lucida tracing. $\times 1.15$.

not have been readily distinguishable from a culture of Class III d. Cultures showing a central aggregate have not been obtained since July 1929, when the series had been under investigation for a year. It seems likely that the capacity to produce them has been lost as a result of continued subculturing.

III c. Cultures producing pink sclerotia. In early stages these cultures are only distinguished from those of Class III b by slightly stronger growth and less tendency to form curved main hyphae. Conidiophores begin to form after some ten days, and arise in tufts from small dense areas on the surface of the mycelium, or from localized spots in the scanty aerial mycelium; a day or so later rudiments of sclerotia appear on the medium,

about half-way between the centre and the periphery. Some of the rudiments soon assume a definite outline, and blacken completely, requiring maybe a month to complete the process; others, distinguished by a rather vague outline, give rise to thin sclerotia which remain brown or pink for



TEXT-FIG. 4. A culture of Variant III b grown in May 1929. The central aggregate is not well defined, radiation is very vaguely shown, and the sclerotia are black. Camera lucida tracing. $\times 1.15$.

months. These abnormal sclerotia may occur scattered among the dark ones, but they generally form in patches, either close to the edge of the medium or in ill-defined sectors. The latter are areas of weak growth; in young cultures they may be distinguished by their slower spread and general thinness, and in mature cultures they bear fewer conidiophores than are present on areas carrying black sclerotia. A number of cultures were started from pink sclerotia, in the hope that cultures could be obtained in which such alone developed; this hope was not fulfilled, for the cultures either resembled the parents or gave cultures belonging to III d.

Inspection of Table IV suggests that the tendency to the production of pink sclerotia is becoming weaker.

III d. Cultures differing from the stock strain in little but general weakness of growth. Young cultures of Class III d are not readily distinguishable from those of Class III c; the growth is slower, and the production of aerial mycelium much weaker than in the corresponding controls. When the margin of the medium is reached a few appressoria may be formed, but such as form are usually light in colour and seldom macroscopic; in the preceding classes appressoria are rarely seen. The formation of conidiophores and of sclerotia proceeds as in III c, but the sclerotia all blacken, and usually do not blacken much more slowly than in control cultures.

TABLE III.

*Analysis of Series III, according to the Age of the Parent Culture.
July 1928 to August 1929.*

Age of parent, days.	Class of resulting Cultures.				
	III a.	III b.	III c.	III d.	III e.
5-10	14	1	1	—	—
11-20	14	1	10	13	2
21-30	7	4	5	11	3
31-40	6	1	5	2	2
41-50	9	5	10	13	5
51-100	3	1	2	6	4
101-137	4	5	3	1	1
Totals	57	18	36	46	17

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TABLE IV.

Analysis of Series III in Periods of Three Months.

Period.	Class of Culture.				
	III a.	III b.	III c.	III d.	III e
1928.					
July-September	2	—	2	6	—
October-December	33	7	13	17	8
1929.					
January-March	12	6	2	3	2
April-June	4	4	13	5	7
July-September	6	1	6	15	21
Totals	57	18	36	46	38

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Cultures of III d form an important fraction of the series; they have appeared quite erratically among other and more aberrant cultures. Transfers from them have yielded similar cultures, or cultures difficult to distinguish from the normal form.

III e. Nearly normal cultures. These cultures have been obtained when conidia have been transferred from specially dense conidial areas in cultures of III d, or when conidia have been taken from tufts on old sclerotia. In addition, most of the cultures of Series III, which were made

during the hot weather in August and September, 1929, could hardly be distinguished from control cultures. They seem to have reverted completely to the original form.

Series IV.

Series IV began with the transfer of a whole light-coloured sclerotium from Culture A. Growth was at first low and loose, but when the culture was nineteen days old the centre of the dish was occupied by a dense cushion of white cottony mycelium bearing a few conidiophores; growth had stopped before the edge of the medium was reached, and sclerotia did not form. At this time conidia were transferred to fresh media, and from these cultures single colonies were isolated.

These cultures, and those which immediately followed them, produced some pink sclerotia, as well as a dense central cushion of aerial mycelium. From one of these cultures four pink sclerotia were taken. They gave cultures, three of which were the starting-points of Series V, VI, and VII, respectively; the fourth was not further investigated. Conidial transfers from the early cultures of Series IV, taken from above pink sclerotia, failed to yield growth. This is comparable with results obtained in other series. On the other hand, conidial transfers from above black sclerotia gave rise to a series of cultures not differing in any important respect from normal. On the whole, these cultures gave more and larger sclerotia than the corresponding controls; but although the series was carried on for a year striking differences were not obtained.

Series V, VI, and VII.

These series began with cultures made from single pink sclerotia taken from an early culture of Series IV. In all three growth was at first low, with very little aerial mycelium; it was noted that the pink sclerotia soon blackened after they had been transferred to fresh media. The surface of the medium was covered in eight to nine days, then a scanty crop of conidiophores formed, a central cushion of aerial mycelium developed, and rudiments of sclerotia became visible. All three cultures ultimately produced so heavy a crop of cottony aerial mycelium that the free space in the dish was filled, and pitchy black appressoria were formed against the under-side of the lid. Sclerotia appeared in all, and some of these remained pink for months; black sclerotia were also formed, a few of which were surrounded by a purple stained area.

From these cultures conidial transfers were made and single colonies isolated; the further history of these lines will be considered separately.

Series V.

Series V is of special interest. One line of development in the series shows, perhaps even more clearly than in Series III, a gradual return to

normality ; another line has resulted in the isolation of a strain distinguished by the production of microconidia, and of considerable stability.

The cultures of Series V fall into the following groups :

V a. Cultures showing weak growth or no growth. As in Series III these cultures have been obtained by transfers from areas bearing pink sclerotia. They do not need further discussion.

V b. Cultures producing nearly sterile, weak, white aerial mycelium. Some of the conidial transfers from above pink sclerotia gave weakly growing cultures which remained nearly or quite sterile, did not produce appressoria, and never formed good sclerotia. One such culture, when twenty days old, produced fourteen small red spots on the surface of the medium, which looked like sclerotial rudiments. These did not change after further observation, and when transferred to fresh media failed to give growth. Another culture, when eighty-six days old, yielded three bright ruby red sclerotia, which appeared normal in structure. One was examined, and two portions of it placed on fresh media ; the others remained unchanged for five months. Of the transfers, both gave weak growth. A few poor conidial groups were seen when the original culture was 141 days old, and transfers from these gave the microconidial strain.

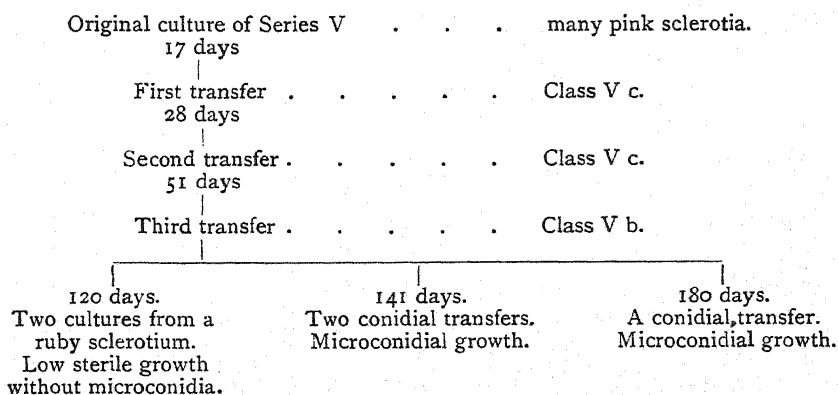
V c. Cultures producing radial streaks of pink sclerotia. One of the subcultures from the original culture of the series yielded a slow growing colony, on which conidiophores did not appear until the fourteenth day. The aerial mycelium was distinctly pinkish, and the sclerotial rudiments, which appeared with the first conidiophores, were also pink. The larger sclerotia, which lay towards the centre of the dish, darkened in ten days ; those which lay towards the periphery in radiating lines (Pl. XXXVII, Fig. 5), not ending in a point as in Series III, remained pink. Conidial transfers made when the culture was twenty-eight days old gave rise to two more cultures showing the same phenomena ; transfers from these failed to reproduce the condition ; one gave a culture of class V b, the others gave V d.

V d. Cultures with pink sclerotia. In all, twenty-two cultures of Series V developed some sclerotia which failed to blacken, remaining pink or brown. They appeared most abundantly in the earlier part of the work. Those obtained in the autumn of 1928 were characterized by slow initial growth (one to two days behind the controls), the production of but little aerial mycelium, the presence of a raised edge of pink hyphae at the advancing edge of the mycelium, and the abundant production of pink appressoria on the side of the dish, and of very black appressoria in a strong ring where the lid rested on the side of the dish. The crop of pink appressoria was so strong that the cultures showing this character could be identified as they stood in the stacks. During 1929 the formation of pink appressoria, pink sclerotia and pink aerial mycelium gradually fell off, so that there was gradual merging into cultures of the next class.

V e. Cultures producing a weak crop of conidiophores. Most of the cultures of Series V fall into this class (Pl. XXXVII, Fig. 6). The early cultures began very like those which produced pink sclerotia, and only differed from them in the behaviour of the sclerotia. These were rounded, and definitely pink at first, but they usually blackened completely in about ten days: the aerial mycelium was pinkish, and pink appressoria formed freely. As time went on, the tendency to show a pink tinge in early stages became weaker, so that the later cultures of the class differed from control cultures in slightly slower growth, in a tendency to form rounded rather than radially elongated sclerotia, and in the general production of a thin crop of conidia. In this condition, the cultures may be readily identified on inspection, but it is impossible to state any distinct character which separates them from control cultures. During the hot weather these cultures gave much heavier crops of conidia, with a good deal of brownish aerial mycelium, and could not be distinguished from controls, which at that time also showed a tendency to give pink appressoria. These cultures seem to have reverted completely to the original form; they appear in Table V under V f.

V g. Cultures of dense white character. Three transfers made from cultures of Class V e during hot weather gave colonies like those of I b. This is not altogether surprising, for this variant is the prevailing form in Series VI and VII, which arose from pink sclerotia formed in the same culture as that which gave Series V. As I b yields but a scanty crop of conidia, it does not seem possible that these three cultures can have arisen from contamination; had this been the case it is much more likely that either the stronger normal form or the ubiquitous *Penicillium* would have appeared.

V h. The microconidial strain. The origin of the microconidial strain is as follows:



It is clear that the microconidial strain has distinctly aberrant cultures in its line of descent.

Once microconidia were obtained difficulty was not experienced in culturing the strain, for they germinate readily.

Allowing for slight individual differences the thirty-nine cultures of V h. may be included under the following general description.

Both mycelial and conidial transfers have been made, the former from young cultures, the latter from older ones. Germination is visible by the second day; a low and silky colony is formed (Pl. XXXVII, Fig. 7), covering the medium in about ten days. On media 1–2 mm. in thickness, however, growth may cease before the edge is reached; acid does not appear to be secreted into the medium. A very slight amount of aerial mycelium is formed, and the whole colony is nearly transparent, and appears of the same colour as the medium. Growth is mostly submerged, and branching dense and even. Indications of groups of microconidia are seen when the culture is about three weeks old; the groups are small, and often invisible to the unaided eye, and so dark green in colour as to appear black to the lens. Frequently, but not always, when a colony has reached a diameter of about 4 cm., further growth takes place from the centre, at a higher level in the medium than that occupied by the earlier growth. This gives a layered effect, often a stellate effect, as the layers may run out in triangular patches. The whole has a filmy appearance, quite distinct from any other of the cultures seen in this investigation.

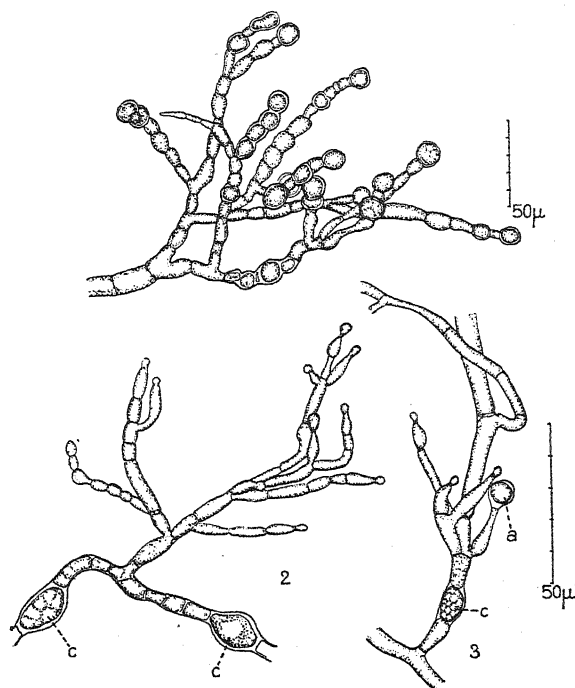
When young the hyphae are of even diameter, but when the culture is a week or so old, the hyphae may be converted into chains of chlamydo-spores, or into chains in which such spores alternate with unaltered hypha; this phenomenon was most frequent in early cultures of the form (Text-fig. 5, 1). A complete morphological investigation of this interesting form has not yet been made; indications have been seen that the chlamydo-spores are not invariably formed by the transformation of existing branches, but often arise on the submerged mycelium from short lateral branches which, in everything but size, recall the sterigmata on which microconidia are formed (text-fig 5, 3 a).

Microconidia are always formed above the surface of the medium. In view of the extensive literature dealing with these structures (Brierley, 6; Istvanffi, 10; and the papers mentioned by these authors), it is unnecessary to embark on a thorough description.

Sclerotia which have remained creamy in colour and translucent for two months were obtained in one of these cultures (Pl. XXXVII, fig. 8). So far, transfers from this culture have failed to yield sclerotia of any kind. In three cultures in which the normal form and the microconidial form were grown together, the latter gave rise to extraordinary sclerotia—whitish, flat, and presenting the general appearance of a piece of dirty ice. The normal form grew around, but not over these sclerotia.

The microconidial form has retained its general characters in culture

from May to September 1929¹; the latest cultures show a little more development of aerial mycelium, less inclination to form submerged chlamydospores, and a tendency to produce successive layers of growth in circular patches, and not in a stellate pattern. These changes may indicate



TEXT-FIG. 5. The microconidial strain, Variant Vh. 1. Chains of chlamydospores, swollen hyphal segments, and almost unaltered segments. 2 and 3. Young stages in the formation of groups of microconidia. 3a shows a stengma which has grown into the medium and formed a chlamydospore. 2c and 3c Chlamydospores on hyphae just beneath the surface of the medium.

a return to more normal conditions. Brooks (8), who obtained an apparently similar form on a special medium, records that it soon reverted to normal.

TABLE V.

Analysis of Series V, in Periods of Three Months.

Period.	Class of Culture.							
	V a.	V b.	V c.	V d.	V e.	V f.	V g.	V h.
1928.								
October-December	12	1	3	12	20	6	—	—
1929.								
January-March	1	4	—	6	11	3	—	—
April-June	1	2	—	4	25	1	—	6
July-September	—	1	—	—	6	34	3	33
Totals	14	8	3	22	62	44	3	39

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¹ See note at end of paper.

Series VI and VII.

The origin of these series is given on p. 845. In both series the dense white form corresponding closely with that obtained in Series I has been obtained. Owing to the tardy formation of conidia by this form only fifty-four cultures have been made in the two series, and, whether fresh cultures have been prepared by means of conidial transfers or by means of pieces of mycelium, the form has appeared in all the cultures. As the form was described in Series I, it needs no further treatment here.

Series VIII.

Series VIII needs but brief comment. It began with one of the whole light coloured sclerotia from Culture A, and the first few cultures produced a good deal of whitish and sterile aerial mycelium. Soon, however, transfers began to give cultures distinguished from control cultures only by the production of a few whitish sclerotia (Pl. XXXVII, Fig. 9). Growth from these gave similar cultures, in which the capacity to form white sclerotia was lessened, and after three months of culture this series was allowed to lapse, as nothing but normal cultures appeared.

CONCLUSION.

The evidence presented in the foregoing pages is sufficient to show that cultures of *Botrytis cinerea*, derived immediately from heated spores, or belonging to a stock originating from heated spores, may present characters distinct from those shown by companion cultures of the normal strain, made at the same time, and grown under the same conditions. During three years of culture, under ordinary laboratory conditions, the normal strain, whether grown from single conidia, mass transfers of conidia, or pieces of hyphae, has not yielded any variant which could be perpetuated in a series of cultures, nor, at an age when the peculiarities were clearly visible in cultures of the variants, have comparable peculiarities been visible in the controls. Although cultures of the normal strain, when started from sclerotia, have yielded subcultures producing heavy crops of sclerotia, it has not been found possible to establish a sclerotial strain in this way, nor to transmit the tendency to cultures inoculated by means of conidia.

In old cultures, and in cultures grown at relatively high temperatures, the normal strain has shown a tendency to produce unusual growths, but these abnormalities could not be transmitted to subcultures: they occurred only under special conditions.

As the normal form has been so constant in its behaviour, it seems unlikely that the variants, which have been isolated and grown side by side

with it, can be explained on the supposition that they are merely normal fluctuations, likely to occur in any series of cultures of *B. cinerea*, grown for a sufficient length of time. Since the variants have been obtained only from cultures with a heated spore in the line of descent, it seems unnecessary to seek for any explanation other than that suggested by the evidence.

The experiments with heated spores have shown the same capriciousness in results noted in the earlier work on *Eurotium herbariorum*. This is in accordance with expectation, for there is no known method of obtaining a standard sample of spores for experimental purposes; the spore contains several nuclei, and its potentialities are unknown. Even were it possible to subject single spores from the same head of conidia to identical treatment, it by no means follows that all would react in the same way. The biologist cannot hope for such constancy in his results as is obtainable by the chemist, who starts from surer foundations. A further difficulty arises in the selection of a standard with which to compare the results of experiments. The only possible way is to secure a strain of the fungus which appears to possess the general characters of the species, to establish its constancy in culture, under the proposed conditions of experiment, and to use it as an arbitrary standard. The choice of the stock strain used in the present investigation seems to be justified by the constancy it has shown during nearly three years of culture: the forms obtained from it may justifiably be regarded as variants of the standard chosen.

Forms resembling the variants described here, and possibly identical with them, have been isolated from naturally occurring material, or have been produced experimentally by workers with *B. cinerea*. Paul (11) records a number of strains isolated from vegetable substrata, and it is clear from the accounts of other investigators that a variety of forms may be obtained from wild material. A variant characterized by the habitual formation of light coloured sclerotia has been described by Brierley (7), and maintained in culture for years; it appeared suddenly, and its origin is obscure. Beauverie (3) produced a white sterile mycelial strain of *B. cinerea* by prolonged exposure of the ordinary form to a comparatively low temperature and great humidity on a poor substratum.

It is impossible to explain how distinct strains isolated from rotting plants came into being, as the history of such material must remain unknown; under natural conditions, however, spores could easily be exposed in sunlight to heating as intense as that used in the experiments described in this paper, and there is no reason to suppose that variants arise only as a result of treatment by heat. The resemblance of artificially induced variants to naturally occurring strains is not surprising: experiment is hardly likely to bring about the appearance of entirely new forms.

A piece of evidence drawn from another organism shows that agree-

ment may exist between abnormalities developed by experiment and abnormalities found in untouched plants. Gain (9) has described the results of exposing the embryos of *Helianthus annuus* to desiccation, followed by severe heating; among the abnormalities noted were some peculiarities in the shape of the leaves. Similar abnormalities were described by the author (1) from an old plant of *H. annuus*, which, so far as is known, was not exposed to unusual conditions. Clearly, in the sunflower, exposure of the embryos to heat is not the only means by which the development of malformed leaves may be induced. The formation of similar abnormalities by plants grown from heated embryos, and by an old plant, may be significant. A parallel exists in another respect between the work of Gain and the present work on *Botrytis*; some of the plants of *Helianthus* flowered, but they did not yield fertile seed. The comparison is not close, but the production of enfeebled conidia by *Botrytis* may be recalled.

Of the variants induced in *Botrytis* four differ notably from the original strain. They are: the dense white form of Series I, VI, and VII, the sclerotial strain of Series I, the variant of Series II, characterized by the free production of brownish aerial mycelium, and the microconidial strain of Series V. The first two are probably permanent, the others possibly so; all have remained constant in culture for months, and all have subcultured cleanly. The remaining variants appear to resemble the transitory variants noted in *Eurotium*; in that fungus they were not subjected to prolonged investigation. When these variants first appeared in *Botrytis* they were sharply distinguished from the normal strain by a tendency to develop a light pink colour in some or all of the young organs, and to yield limited crops of conidia; a few showed unusual grouping of the sclerotia, and all grew weakly in young stages. Many of these peculiarities were indicated in cultures of the stock strain grown at temperatures around 26° C., but from these cultures the special features could not be transmitted to subcultures grown at lower temperatures. The possibility that prolonged culture at a fairly high temperature might yield variant forms has not been tested.

As culture succeeded culture of the transitory variants, their distinguishing features became more weakly expressed; the production of the pink pigment soon diminished; the tendency to weak growth and the development of reduced crops of conidia lasted for more than a year. The general drift back to normal shown by these variants, and analysed in Tables IV and V, is perhaps even more striking evidence that the original changes were due to the disturbing effect of heat on the spores than is the isolation of permanently altered forms. The gradual resumption of the characters of the normal strain, with the slow and orderly disappearance of one distinguishing feature after another, and the accession of strength of growth as the amount of pink colour in the young organs fell off, suggests

that recovery was taking place from the condition of injury set up by the heating of the spore.

The development of a pink pigment in the mycelium, appressoria, and sclerotia of *Botrytis*, appears to be a matter of some interest. The phenomenon has been seen in tufts of aerial mycelium produced by old control cultures of the normal strain, in the aerial mycelium and appressoria of cultures of the normal strain grown at temperatures above those most favourable for development, and in all parts of some of the non-stable variants. A definite connexion undoubtedly exists between the appearance of the pink colour and lack of vigour in the fungus. In the transitory variants most of the sclerotia which remained pink were of indifferent quality, noticeably thin and flat, uneven and vague in outline, and associated with areas of weak mycelial growth; with few exceptions, these sclerotia, in appearance and manner of development, suggested structures which just managed to form and no more. They usually sprouted readily when transferred to fresh media, and always gave rise to colonies in which the presence of a good deal of white, sterile aerial mycelium was a marked character; but a culture started from a pink sclerotium has never given one in which pink sclerotia alone developed. The early growth from pink sclerotia was always weak.

In some of the first cultures of Series V pinkness was visible in the young mycelium; in these cultures a tendency to the arrangement of the sclerotia in radial streaks was often apparent, growth was low and weak, and the crops of poorly defined pink sclerotia were heavy. These cultures were markedly different from the corresponding controls, and in their characters suggested profound disturbance of normal behaviour.

Lack of vigour was also shown, especially in Series III, by the conidia developed over areas bearing pink sclerotia; the conidia often failed to germinate. The weakness in the mycelium of these areas was shown by the poor results often obtained from mycelial transfers. In Series I it was shown that conidia taken from conidiophores above pink sclerotia gave the slowly growing dense white form.

A relationship between the development of pinkness and non-viable spores has been observed in *Syzygites megalocarpus*. In 1928 isolations of this species from material on *Amanita rubescens* yielded weakly growing subcultures: transfers from these grew feebly, and produced a few bright pink sporangia which failed to blacken. The sporangiospores appeared to be normal, except in colour, but they would not germinate.

The development of spores of inferior quality in association with the abnormal appearance of a pink pigment does not seem to have been recorded, but a connexion between pinkness and the slackening of ordinary growth has been noted. Robinson (14) showed in *Pyronema confluens* that formation of reproductive organs from branches of arrested growth is

preceded by the development of a slight pink colour in the aerial branch systems, and Whetzel (15) found in *Sclerotinia Erythroniae* that prolonged culture delayed the appearance of sclerotia, and that these sclerotia, when formed, remained flesh-coloured for a long time. Like the pink sclerotia of *Botrytis*, they often produced a web of white hyphae.

A connexion appears to exist between the presence of the pink pigment and the formation of microconidia. Incubated cultures of the normal form which developed much pinkish aerial mycelium developed microconidia in about five days. The microconidial strain described on p. 847 arose from a line of distinctly aberrant cultures, originally derived from a pink sclerotium, and distinguished by the abundant formation of pink sclerotia. The mycelium of the microconidial strain appears to be faintly tinged with pink.

The nature of the pink pigment is obscure; it has not been possible to obtain enough material to allow of the extraction of the pigment in a useful amount. The methods of Raper and Wormall (12) were tried on pink sclerotia, on the assumption that tyrosin was involved, but the results were negative.

Under ordinary conditions of culture, the appressoria and sclerotia of the normal strain do not pass through a definite pink stage as they blacken, but the experiments with incubated cultures of the normal strain show that the formation of pink appressoria is easily induced by a moderately high temperature. It is not unlikely that the pink substance is an intermediate product in the formation of the darker pigments of mature organs, and that it is stable enough to accumulate, if, as a result of heating, the ordinary sequence of chemical change is disturbed. That the colour is due to an unstable substance is indicated by the failure to establish a permanent variant with pink organs, and by the gradual disappearance of the colour from the successive cultures of Series III and V. The association which has been shown to exist between the occurrence of the pink pigment and general lack of vigour also points to a disturbance of the normal course of development of the fungus.

A deep green pigment is present in the microconidia, and a similar pigment was noted from time to time, especially in the appressoria of incubated cultures. Microconidia have always germinated readily, and conidia taken from cultures with many green appressoria have not shown unusual behaviour.

In addition to these pigments, which hardly appear during the ordinary growth of the normal strain, the brown pigment characteristic of *Botrytis* needs a short consideration. In *B. cinerea* the ripening of the conidia is accompanied by, or sometimes preceded by, the appearance of a brown pigment in the walls of the aerial mycelium. In the series of cultures made during the investigation all stages have been seen from the

white and sterile aerial mycelium through one which is distinctly brown and bears some conidiophores, and the definite brown condition in the normal sporing form, to the deeply-stained and imperfectly-formed conidiophores sometimes seen in Variant I b. It is probable that the heavily stained hyphae observed in cultures of the normal form at 30° C., and in the non-viable form obtained from singed spores, are further stages in this progression. Apparently the formation of conidia and the development of pigmentation in the walls of the aerial mycelium are both indications of a definite condition of equilibrium; when this is disturbed beyond certain limits one or both of the associated phenomena may fail to appear. It seems too that a relation exists between the formation of conidia and of sclerotia and the staining of the medium. Cultures with heavy crops of one or both of these structures seldom show much staining of the medium. When, however, much sterile aerial mycelium is present, a condition which is usually associated with a poor development of sclerotia, and a limited production of conidia, patches of deep purple or black stain usually underlie the mycelium. This condition is specially well shown by the variant of Series II; in variant I b, which forms few conidia, and seldom forms sclerotia, small blackish patches of stain occur freely beneath old colonies. In all the cultures considered, the conditions of growth have been the same; the differences observed are characters of the variants. The details briefly reviewed here are interesting, as they suggest a possible connexion between the retention of the products of metabolism within the fungus, the development of pigmentation, and the subsequent appearance of the organs of multiplication, though they do not indicate any rigid causal relation between these phenomena. The consideration of the staining effects also indicates that one result of exposing spores to heat may be to alter the permeability of the protoplasm, with noteworthy effects upon the general appearance and development of the cultures.

This work has extended the results obtained with *Eurotium*, and so confirms the earlier work. Recently, similar experiments have given successful results with *Thamnidium elegans*, and carried the investigation into the *Phycomycetes*: this will form the subject of a future communication. But although changes have now been obtained in three species by exposing their spores to heat little is yet known of the mechanism of the changes. At present the accumulation of more evidence is held to be of greater importance than any premature attempt at explanation.

SUMMARY.

1. Conidia taken from a strain of *B. cinerea* known to be constant in culture were exposed to high temperatures and transferred to culture media. Of 520 cultures inoculated with heated conidia, 424 failed

to develop growth, 12 gave rise to strongly modified colonies, 64 yielded slight variations, and 20 could not be distinguished from control cultures.

2. The most definite variants arose in cultures showing but few germinations. In three instances a blackened stunted form was obtained, resembling colonies of the normal strain when grown at 30° C.; this form could not be transmitted to subcultures. One culture developed a low whitish growth, not further investigated at the time; a similar form is described as Variant I b. Eight cultures produced some normal sclerotia and a few sclerotia which failed to blacken.

3. From a colony known to have arisen from the only spore which survived heating at 66° C. sclerotia were transferred to fresh media after they had remained light coloured for 105 days. They gave cultures distinguished by a tendency to produce a good deal of loose, white aerial mycelium, few conidia, and few sclerotia. This type of culture was only seen in the early stages of the work; before long, new forms with at least some degree of permanence appeared in subcultures, and were isolated.

4. Three variants have been maintained in culture for thirteen months, having passed through about fifty sets of subcultures. Of these variants one produces a low white mycelium with few conidiophores and rare sclerotia: the second develops a moderate crop of conidia, much nearly sterile aerial mycelium, and no sclerotia: a third forms many sclerotia with few conidia.

5. Two series of unstable variants have been investigated. The early cultures of both were distinguished by rather weak growth, associated with the production of pinkish sclerotia in radiating groups; in one series pink mycelium and pink appressoria were also noted. During thirteen months of culture the abnormal features have slowly disappeared and the normal form has been resumed; the reversion seems to have been hastened by the hot weather in the summer of 1929.

6. From one series, through a line of cultures forming many pink sclerotia, a microconidial strain has been isolated; the microconidia germinate readily, and the strain has retained its characters in culture for nearly six months. One culture of the microconidial strain produced white sclerotia, but a strain characterized by the production of white sclerotia only has not been observed.

7. There is evidence of an association between the occurrence of pink pigmentation in the fungus and weakness of constitution; the pigment has not been investigated, owing to lack of sufficient material.

8. The normal pigmentation of *B. cinerea* is briefly discussed.

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October, 1929.

ADDENDUM.

The foregoing paper was written in the autumn of 1929. Since then, the cultures have been carried on and the position is now as follows:

(a) Variants I a, I b, V g, and V h, have retained their characters unchanged.

(b) Variant II has reverted to the normal form.

(c) The normal form continues steady in behaviour.

September, 1930.

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EXPLANATION OF PLATE XXXVII.

Illustrating Dr. Barnes's paper on Variations in *Botrytis cinerea*, Pers.

The cultures were grown on Reidemeister's medium in Petri dishes 9.2 cm. diameter. Figs. 1, 2, 3, 5, 6, 7, and 8 show the upper surface of the culture; Figs. 4 and 9 were taken through the bottom of the dish.

The microphotographs are from living cultures six days old. In them the diameter of the hyphae is exaggerated by moisture lying on the surface of the medium.

Fig. 1. Colony of the normal strain, showing the even turf of conidiophores.

Fig. 2. Variant I a.

Fig. 3. Variant I b. The mycelium is sterile.

Fig. 4. Variant III b. The contraction of the central aggregate has caused two cracks to form in the medium. The outermost sclerotia of the radiating groups remained pink.

Fig. 5. Variant V c. The radiating streaks of pink sclerotia are clearly seen, as well as vague radiating patches of aerial mycelium; this was pink when young.

Fig. 6. Variant V e. Tufts of conidiophores occur on the sclerotia, in addition to the scanty crop of conidiophores on the aerial mycelium.

Fig. 7. Variant V h. Culture of the microconidial strain, six days old.

Fig. 8. Variant V h. Culture of the microconidial strain with whitish sclerotia. Material had been removed from the three dark patches.

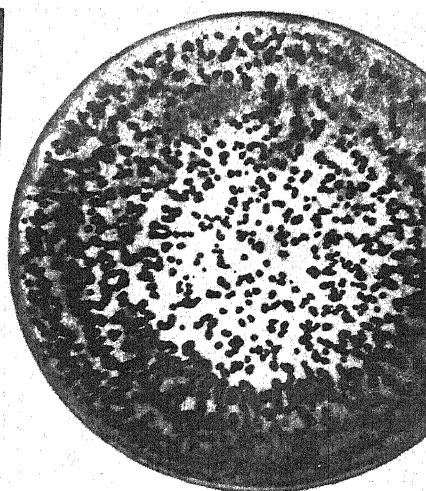
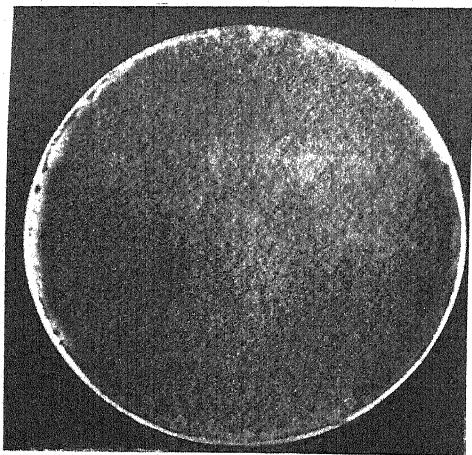
Fig. 9. The variant of Series VIII. The small white sclerotia remained unchanged from October 1928 to July 1929, when the culture was preserved.

Fig. 10. Portion of the edge of a young normal colony. $\times 26$.

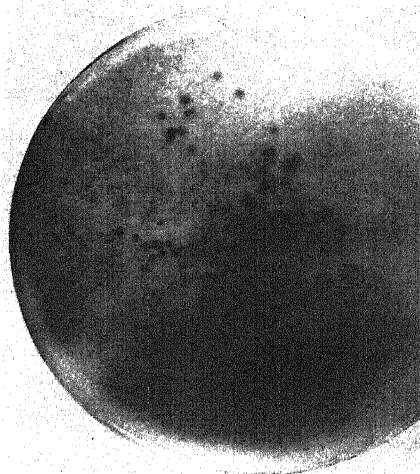
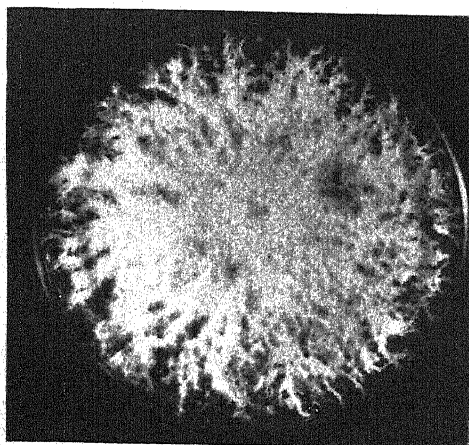
Fig. 11. Portion of the edge of a weakly growing colony of Series III, showing curvature of the hyphae. $\times 26$.

Fig. 12. Portion of the growing edge of Variant I a. $\times 26$. In Variant I b the short lateral branches are more crowded.

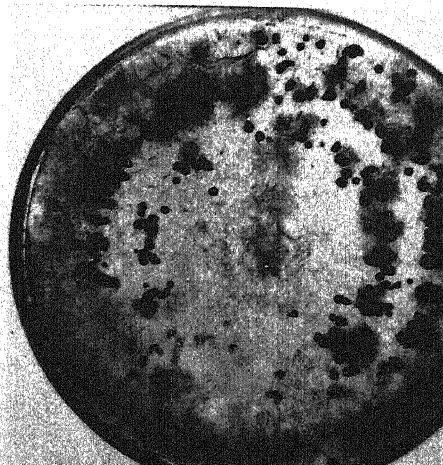
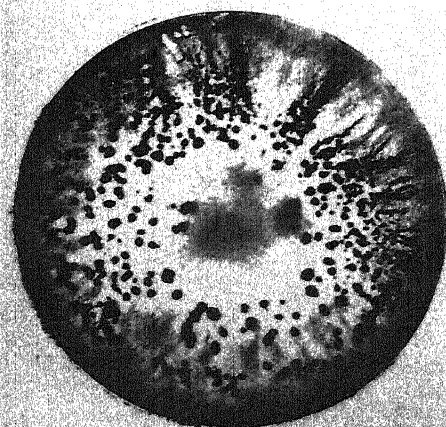
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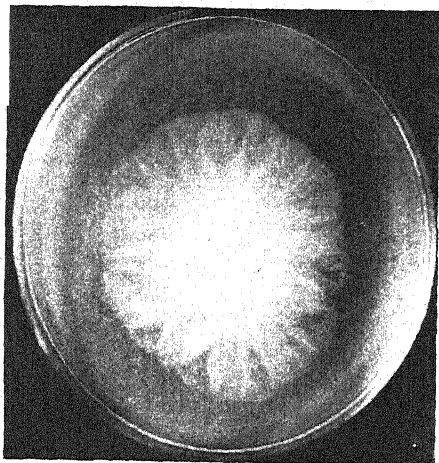
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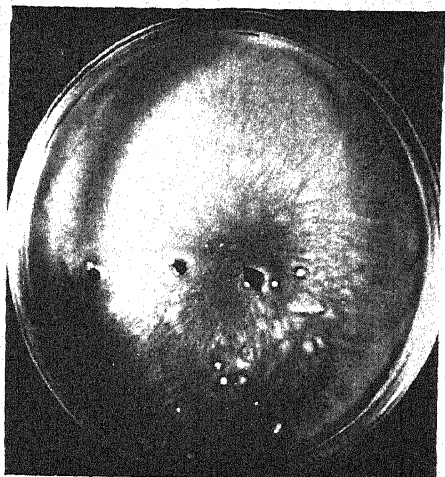
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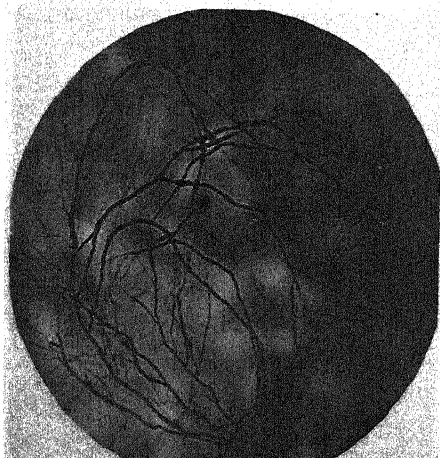
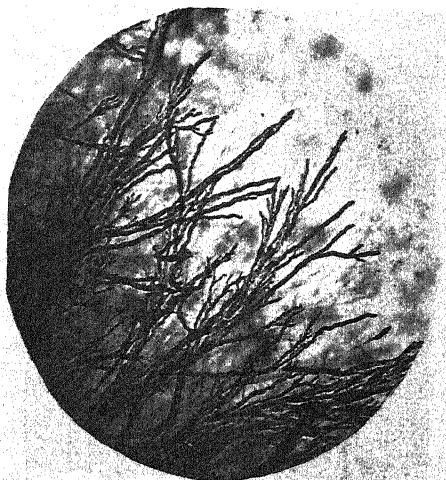
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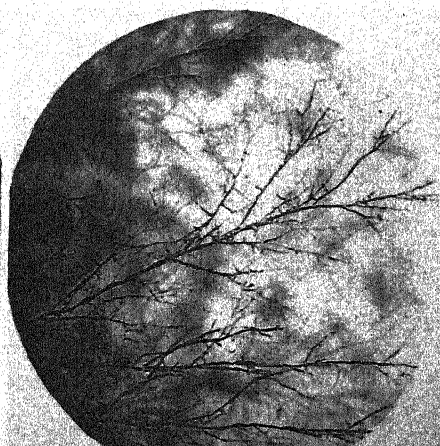
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12



Increased Scion Vigour Induced by Certain Foreign Root-stocks.

BY

W. A. ROACH.

(*Department of Insecticides and Fungicides, Rothamsted Experimental Station, Harpenden.*)

With Plate XXXVIII and one Figure in the Text.

IN the course of grafting experiments made by the author at the Rothamsted Experimental Station, and designed to throw light on the causes of the limitation of certain parasites and symbiotic bacteria (2, 3) to definite host plants, combinations were observed in which the scion made better growth on a foreign root-stock than on its own. These observations seem sufficiently interesting to be collected together in spite of the fact that they were made incidentally to other work, and so are not as complete as could be desired for the present purpose.

I. WOODY NIGHTSHADE ON POTATO.

In the summer of 1922 a number of equal-sized cuttings taken from a single plant of woody nightshade (*Solanum Dulcamara*) were rooted in moist soil in 10-in. pots; of these rooted cuttings five were selected as being as uniform in size, vigour, &c., as possible. Two of these were set aside as controls; the other three were cut off just above soil-level and grafted on single-stem potato (*S. tuberosum*) plants which had been obtained by tearing from the parent tuber a single rooted shoot as soon as leaves appeared above soil, and planting it in a 10-in. pot.

The grafts took readily and, in spite of starting off shorter because of the bits of stem removed in the operation of grafting, they soon outgrew the two controls. In Pl. XXXVIII, Fig. 5, are shown the two control plants and one grafted one. The leaves of the grafted plants were three or four times the area of the control ones and of a much more luxuriant appearance. The axillary bud of almost every leaf on the grafted plants developed into a strong shoot, whereas the ungrafted plants were hardly branched at all. The girth of the grafted plants soon exceeded that of the control ones.

No marked differences in date of flowering or fruiting was noticed. The condition of the mature plants is shown at the top of Plate XXXVIII;

Fig. 1 is of two control plants, and Figs. 2, 3, 4 each show a single grafted plant. The two control plants were taken together. The marked increase in size of top due to grafting on potato is well seen in the illustrations.

The grafted plants were cut off just above the graft, and the controls at a corresponding height above soil-level. The weights of tops were:

Grafted plants	11 grm.	19 grm.	12 grm.	Average = 14 grm.
Control plants	5 "	7 "		" = 6 "

Hence the tops of the grafted plants were on the average more than twice the weight of the ungrafted ones. The grafted plants also produced potato tubers below ground, making the increased vigour of the top the more remarkable.

Potato on woody nightshade. At least a dozen grafts were made of potato on woody nightshade, in which apparently satisfactory organic union took place. Even when dry sand was held round the stem to allow of natural tuber formation the potato scion remained very stunted.

II. *VICIA FABA* (BROAD BEAN) ON *V. NARBONENSIS* GRAFTS.

In an attempt made with Dr. J. Davidson to study the nature of the resistance of *Vicia narbonensis* and the extreme susceptibility of *V. faba* to aphid attack, a number of grafts between these two plants were made in order to ascertain the effect on the aphides of grafting a foreign root-stock to their host plant. The main results of this work have not yet been published. The following observations were made incidentally during the course of the experiments.

1924 Experiment.

Seeds were sown in soil in 10-in. pots, three seeds to a pot. At the time of grafting the seedlings were thinned to one per pot, the thinning being done so as to give as uniform a set of seedlings as possible. The *V. narbonensis* seed was sown two or three days before the *V. faba* seed to counteract somewhat the difference in circumference of the stems. Cleft grafts were made as described elsewhere (2, 3) when the first foliage leaf was opening.

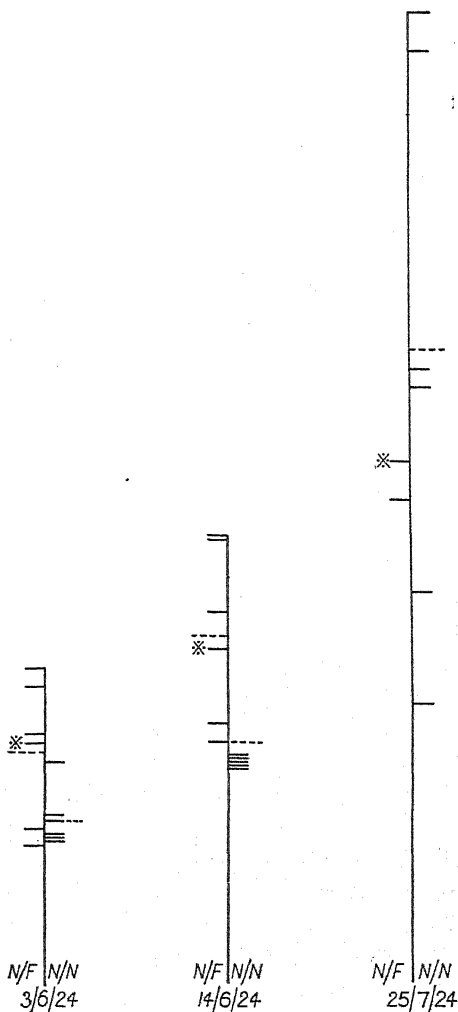
The heights of the plants are shown in the text-fig. The grafts were made on 23/5/24. By 3/6/24 the plants grafted on *V. faba* had outstripped the ones grafted on *V. narbonensis* root-stock, the average height of the one (12.6 cm.) being 1.4 times that of the other (8.8). On 14/6/24 the average heights were 18.8 cm. and 13 cm. respectively, the plants on *V. faba* again being 1.4 times as high as those on their own roots.

Unfortunately, before the next records were taken, on 25/3/24, four plants had died, leaving only two *V. narbonensis* on *V. faba* grafts alive

and healthy, but all six self-grafted *V. narbonensis* plants were healthy. Their average height of 34 cms. was well above that of the one marked *, i.e. 28 cm., which on the two previous occasions was almost exactly an average specimen of the *V. narbonensis* or broad bean grafts. This supports the visual impression that a week or so previous to the day when the final record was taken, before any of the *V. narbonensis* on *V. faba* plants had died, they had been overtaken by the self-grafted *V. narbonensis* plants.

Flowering. On 16/6/24 only one self-grafted *V. narbonensis* plant showed any signs of flowering; in it only the purple tips of flowers were showing. Of the *V. narbonensis* on *V. faba* plants, however, two had one flower each nearly open, two others had one each fully open, and the other two had two flowers each fully open.

Foliage. The foliage of the *V. narbonensis* on *V. faba* plants was of a distinctly yellower colour and of a 'softer' appearance than the self-grafted *V. narbonensis* plants. This yellowing in the bottom leaves gave place to actual browning and final withering, even in plants which were less than half-grown, whereas the self-grafted *V. narbonensis* ones never showed the yellowing, and their bottom leaves only became brown and dry some weeks after the plants had reached their full height.



TEXT-FIG. Heights of *Vicia narbonensis* on *V. faba* (N/F) and self-grafted *V. narbonensis* (N/N). The grafts were made on 23/5/24. Averages represented by dotted lines.

1925 Experiment.

When a similar experiment was carried out in 1925 no plant measurements were taken until the end of the experiments because of the risk of

damaging or disturbing the aphides; inspection of the plants, however, was sufficient to confirm the deductions drawn from the 1924 experiment, viz. the *V. narbonensis* on *V. faba*, plants at first grew faster than the self-grafted *V. narbonensis* ones, but later they lost their lead. The appearance of the 1925 plants almost suggested that, apart from disease which tended to attack the *V. narbonensis* on *V. faba* plants at the graft unions and at soil-level, they would have been slightly shorter when mature than the self-grafted ones. The *V. narbonensis* on *V. faba* plants flowered earlier than the self-grafted *V. narbonensis* plants, and again were the same curious colour as the 1924 plants.

V. faba on *V. narbonensis* grafts. *V. faba* took readily when grafted on *V. narbonensis*, and remained healthy-looking, but in no single graft did one approach in size a self-grafted one.

III. LUPIN ON BROAD BEAN GRAFTS.

In 1924 Mr. H. G. Thornton and the writer commenced a series of experiments to determine whether the specific relationship existing between a leguminous plant and its nodule-forming organism is influenced by grafting a foreign top on the plant. It was noticed that lupins grafted on broad bean root-stocks grew better than when self-grafted, and even than ungrafted lupin plants.

In Pl. XXXVIII, Figs. 8 and 9, are shown two pairs of plants, the left-hand one in each being an ungrafted lupin plant, and the right-hand one a lupin grafted on broad bean. The difference due to grafting on broad bean hardly needs comment, increase in stem girth, amount of branching, and size of leaves being well seen.

In 1927 a more extensive series of experiments was carried out. The plants were grown in sand and watered with culture solution. Each pot contained one ungrafted broad bean, one self-grafted broad bean (1 only had died), 2 broad beans on lupin plants (only 7 survived to the end of the experiment), 2 lupins on broad bean plants (15 survived), one self-grafted lupin plant (6 only survived), and one ungrafted lupin plant (all of these survived).

The following notes were taken when the plants were washed out to search for nodules on the roots (see Pl. XXXVIII, Figs. 6 and 7, in each of which from left to right are two lupins grafted on broad bean plants, one self-grafted lupin, one ungrafted lupin). As none of the lupin roots had nodules on them, for fairness in comparison and simplicity of presentation only those broad bean roots which remained uninfected are considered, although the inclusion of the infected plants would make little, if any, difference to the general conclusions.

Every self-grafted lupin was smaller than its ungrafted fellow. Twelve

lupins on broad bean plants (including one with slightly-diseased roots) were each larger than the corresponding ungrafted lupins; one more was larger than the corresponding self-grafted lupin, but smaller than the ungrafted one. Two, including one with slightly diseased roots, were smaller than the corresponding ungrafted lupin, but larger than all the self-grafted lupins. (The self-grafted lupins actually corresponding to them had died.)

Broad Bean on Lupin Grafts. Broad beans, when grafted on lupins, remained stunted, although, as far as could be judged from a naked-eye examination, satisfactory organic union had taken place.

DISCUSSION.

Darwin, in 'Animals and Plants under Domestication' (vol. i, p. 147), states: 'According to Mrs. Abbey, grafts or buds generally take on a distinct variety or even species . . . with greater facility than on stocks raised from seeds of the variety which is grafted; and he believes this cannot be altogether explained by the stocks in question being better adapted to the soil and climate of the place. It should, however, be added that varieties grafted or budded on very distinct kinds, though they may take more readily and grow at first more vigorously than when grafted on closely-allied stocks, afterwards often become unhealthy.' Darwin was referring to woody grafts, but it is interesting to note how closely the results of grafting *V. narbonensis* on *V. faba* are in agreement. There was little, if any, evidence in the lupin on broad bean grafts to suggest a loss of health in the mature plant. Certainly the woody-nightshade scions grafted on potato retained their enhanced vigour to the end of the season. Any more detailed comparisons of the foregoing results with those of woody grafts, as summarized by Hatton (3), though tempting, would hardly serve a useful purpose at this stage.

SUMMARY.

Three examples are described of a scion being more vigorous when grafted on a foreign root-stock than on its own.

Woody nightshade (*Solanum Dulcamara*) attained more than twice its normal weight when grafted on potato (*S. tuberosum*), and assumed a more branching habit. *Vicia narbonensis* at first grew abnormally tall when grafted on *V. faba*, and then was overtaken by the self-grafted plants, becoming unhealthy before reaching maturity; flowering was earlier.

Lupin when grafted on broad bean was of greater girth and height than when on its own roots.

In the three reciprocal grafts the root-stock had a dwarfing effect.

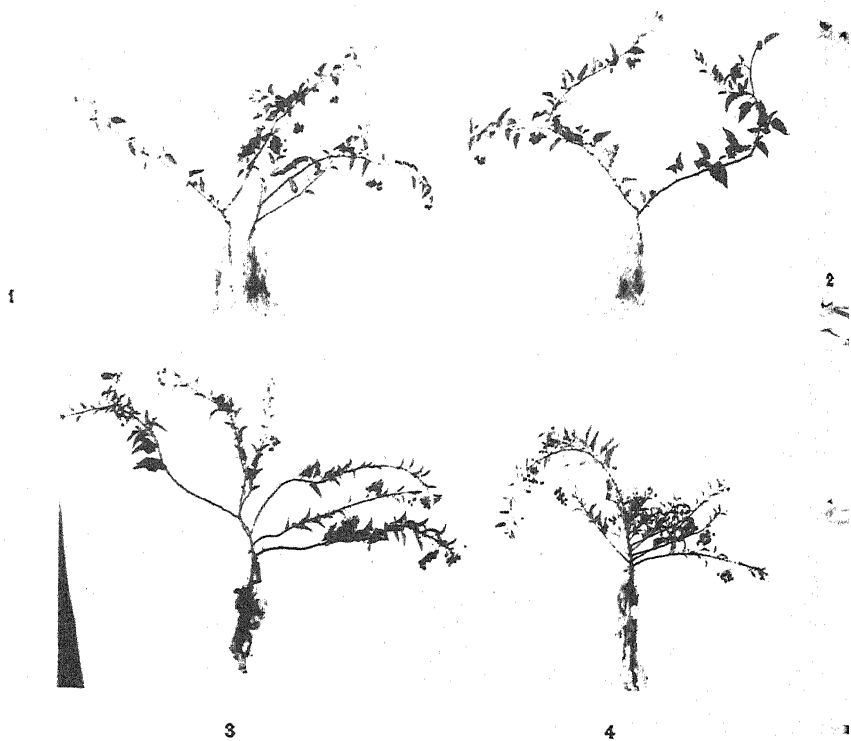
EXPLANATION OF PLATE XXXVIII.

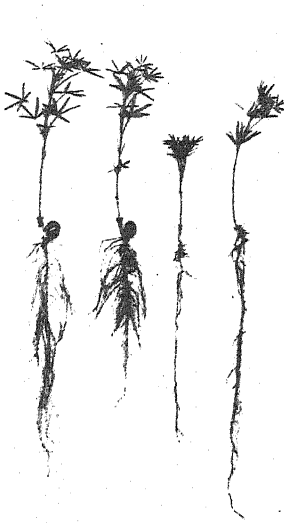
Illustrating Dr. W. A. Roach's paper on Increased Scion Vigour Induced by Certain Foreign Root-stocks.

- Fig. 1. Two ungrafted woody nightshade (*Solanum Dulcamara*) plants.
 Figs. 2, 3, 4. Single woody nightshade grafted on potato plants.
 Fig. 5. Early stage of the above plants. Left to right: two ungrafted woody nightshade plants, one woody nightshade grafted on potato plant.
 Figs. 6 and 7. Left to right: two lupins grafted on broad beans, one self-grafted lupin, one ungrafted lupin.
 Figs. 8 and 9. Left: Ungrafted lupin. Right: Lupin grafted on broad bean.

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3. ——— : Immunity of Potato Varieties from Attack by the Wart Disease Fungus, *Synchytrium endobioticum*, Schilb (Perc.). Ibid., xiv, pp. 181-92, 1927.





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9

The Rootlets of 'Amyelon radicans', Will.; their Anatomy, their Apices and their Endophytic Fungus.

BY

A. C. HALKET.

With Plates XXXIX and XL and six Figures in the Text.

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I. INTRODUCTION.

OUR knowledge of fossil plants is based on the examination of heterogeneous collections of fragments preserved in the rocks, consequently, though much valuable information has been obtained concerning structure, very little is known of the method of growth, or of the biological relationships of the plants.

It was a matter of considerable interest therefore when, in a preparation made from a nodule from the British Coal Measures, a longitudinal section of the apical portion of a rootlet was found, which not only showed the structure of the root-apex, but also had fungal hyphae present in its cortex, and forming a definite 'fungal zone' round the stele. Further search revealing the presence of many sections of similar rootlets in the same preparation, and also in other preparations available, their examination was undertaken.

The data obtained as a result of this examination form the subject matter of the present paper, in which the anatomy of the rootlets, the structure of their apices, and the nature and distribution of the endophytic fungus are described.

After most of the data had been obtained, an examination of the preparations investigated by Mr. T. G. B. Osborn, proved that these rootlets were the same as those described by him in his paper, 'The Lateral Roots of *Amyelon radicans*, Will., and their Mycorrhiza' (17). Nevertheless the additional information, which the better preservation of the tissues of the rootlets now studied has enabled the writer to obtain, is thought to be of sufficient value to justify a further description of these interesting rootlets.

II. THE MATERIAL.

Sections of the rootlet were found in many preparations made from calcareous nodules from the British Coal Measures. The section of the root-apex which first arrested attention was found in a preparation supplied by Mr. Lomax from the Lomax Palaeo-botanical Laboratories of the Lancashire and Cheshire Coal Research Association. This preparation was one of a series of five (Bedford College Collection, 51-55) cut from one calcareous nodule which was obtained from the Upper Foot Mine, Shore, Littleborough. Three other preparations containing numerous sections (Bedford College Collection, 24, 25, and 26) were used which were obtained from the same locality. Other preparations in the Bedford College Collection yielded useful information, the most important of which were slide 56, locality Dulesgate; slide 57, locality Oldham; slides 30 and 31, locality Shore; and the series of slides 154-9, locality Shore.

Through the generosity of Professor F. W. Oliver observations made on this material were confirmed, and additional information was obtained, by a study of preparations in the University College, London, Collection.

The most useful of the University College preparations were two series of slides. The first series consisted of six rock-sections (University College, London, Collection, Q 19, A 1-6) cut from one 'coal-ball' in which the plant tissues were very beautifully preserved. The second

series consisted of eight sections¹ (Professor D. M. S. Watson's Collection, A 283 (1-8)) of a nodule from Shore or Oldham. This second series was supplemented by preparations A 275 and A 282, made from material from the same locality.

The greater number of the sections of the rootlets being found in the three series of consecutive preparations, the nodules from which these were cut will, for the sake of brevity, be referred to as nodules A (Bedford College Collection, slides 51-5), B (University College, London, Collection, slides Q 19, A (1-6)), and C (Professor Watson's Collection, slides A 283 (1-8)).

III. THE ANATOMICAL STRUCTURE OF THE ROOTLETS.

The well-known similarity in the anatomical structure of roots makes the identification of detached roots a difficult matter. In the case of fossil roots the difficulty is increased by the state of preservation of the plant tissues, which is seldom so perfect that the details of cell structure can be observed. Also the material, imperfect as it is, is limited in amount, and many of the sections available are not cut in the most useful plane. An added difficulty lies in the possibility that the fossil root under investigation may belong to a plant whose other parts are still unknown.

A knowledge of the details of the anatomical structure of a fossil root provides, in many cases, the only available criteria for the identification of the individual roots sectioned, and for the establishment of their connexion with other parts of the plant.

The anatomical structure of the rootlets will therefore be described before the structure of the root-apices is discussed, and an attempt is made to elucidate the relations that existed between the endophytic fungus and the cells of the root.

Furthermore, the anatomical structure of the tissues of the rootlets differs in some respects from the description generally given of *Amyelon radicans*, so it is necessary to define their anatomical characteristics before reasons are given for their identification with the rootlets of the well-known Cordaitean root, *Amyelon radicans*, Will.

Many sections of the rootlets are present in the preparations, the greater number of which are cut transversely, but some are cut obliquely and others longitudinally. Ample material is therefore available for the determination of their anatomical structure.

The rootlets vary in age, as can be seen from the degree of differentiation of their tracheides, and in size, but all have certain distinguishing characters.

¹ These preparations from Professor Watson's collection were some of those examined by Professor Osborn. They are at present lent to the Botanical Department of University College, London, and are placed in the University College, London, Collection.

The detailed structure of the tissues of the root will be described under the two regional divisions, the cortex (including the outside layer of cells) and the stele.

The relative dimensions of cortex and stele are shown in the photographs reproduced in Pl. XXXIX, Figs. 1 and 2. The proportions shown are those found in the majority of the rootlets examined, though some variation was observed, some of the roots having a relatively larger cortex.

(i) *The Cortex.*

The cortex is composed of thin-walled parenchymatous cells slightly elongated in the direction of the longitudinal axis of the root. It is not obviously differentiated into layers, the cells being similar in size and in general appearance. However, many of the transverse sections are slightly flattened, and have the cells of the inner layers somewhat crushed, so the walls of the cells in the inner region may have been slightly thinner and less firm in texture than those of the two or three peripheral layers. That this was probably the case is also suggested by the partial destruction of the cells of the inner cortex of the roots in nodule C, in which the plant tissues are less well preserved. Small spaces can be seen between the cells of the inner region, but intercellular spaces are not easily distinguished in the outer layers.

The distinction between the two cortical regions appears to be greater in the roots with a comparatively wider cortex, in these the cell-walls of the inner region appear to be more delicate, and the intercellular spaces somewhat larger.

Fungal hyphae were found, with a few exceptions, in all the roots examined. The mycelium is present in greatest abundance in the inner part of the cortex, and many of the cells are so full of dark contents that a region is distinguishable round the stele analogous to the 'fungal zone' found in many mycorrhizae. In the well-preserved roots the hyphae do not appear to have affected the cell-walls which, though often slightly crumpled, are apparently uninjured.

The presence of a definite 'fungal zone' in the inner cortex, comparable to that found in the roots of many living plants possessing endotrophic mycorrhizae, is perhaps the most interesting feature of these fossil rootlets, and a detailed description of the fungus is given in a later section of the paper.

The cells of the peripheral layer, the epidermis, are not markedly different from those of the deeper layers; they are not quite uniform, some are smaller in transverse section than their neighbours.

Root-hairs were apparently rarely developed. Careful search was made of numerous root sections, but root-hairs were only found in two cases, and one of these was in a transverse section of a very small root of

doubtful identity. One may therefore conclude that root-hairs were not developed under the conditions in which the rootlets examined lived, or it may be, as is stated by Strasburger (34, p. 343) and others, to be characteristic of the roots of some gymnosperms, that these rootlets did not normally form root-hairs.

The innermost layer of the cortex, the endodermis, is differentiated from the rest of the cortex, and forms a distinct sheath round the stele. The cells differ from the neighbouring cortical cells in their smaller size, and in the nature of their walls. The nature of their walls varies with the age of the root. In a few transverse sections of young roots, dark thickenings, comparable to the 'dots' formed by the distinctive 'Casparian strip' of a typical endodermis, were seen on the radial walls of the cells. While in slightly older roots the radial walls of the cells are darker in colour, and appear slightly thicker than the tangential walls. In most of the root sections, however, the walls of the cells are uniformly dark brown, darker than the walls of the adjacent cells. Comparison with the roots of living plants suggests that the endodermis of this fossil root developed in a similar way to that of some modern roots passing through both the primary and secondary stages of development (Kroemer (12)). The endodermal cells had the characteristic 'Casparian strip' on their walls when young, but later, when mature, had all their walls uniformly thickened and dark in colour, which suggests suberization as in modern roots. No cells with thinner walls, comparable to 'passage cells', were seen in the endodermis.

(ii) *The Stele.*

The xylem and the phloem both show characteristic features.

The central strand of xylem is generally diarch, though occasionally it is triarch. An indication of the relative frequency of diarch and triarch rootlets is given by the proportional numbers present in one of the preparations examined, in which fifty-seven transverse or slightly oblique sections were noted, fifty-three of these were diarch and four triarch. The rootlets differ in age in the same preparation, for the degree of lignification of the tracheides varies; in some sections two groups of xylem elements can be seen with thin-walled undifferentiated cells between them, while in most sections the xylem is further differentiated, and a diarch plate is formed. The diarch plate is narrow, as a rule it is one cell in thickness.

In some sections the preservation of the tissues is so good that the sculpturing on the tracheal walls can be seen. The tracheides of the protoxylem are spirally thickened, while those of the metaxylem have their walls covered with closely-packed bordered pits (Pl. XXXIX, Fig. 4). Many of the pits are imperfectly preserved, but the form of the bordered pits can be clearly seen in some of the tracheides. The opening of the pit is an

elongated ellipse, the long axis of the ellipse being always at the same angle to the long axis of the tracheide. These tracheides of the metaxylem are similar to those which occur in the primary xylem of many gymnosperms, as, for example in the roots of *Lyginopteris*, the roots and leaves of Cycads, and the leaves of *Cordaitea*. The close resemblance to the tracheides of Cordaitan leaves can be seen by comparing the pitting on the tracheide photographed (Pl. XXXIX, Fig. 4) with that figured by Stopes (32), Pl. IX, Fig. 6).

The pitted tracheides were formed immediately after the spiral tracheides of the protoxylem, no tracheides with pitting of an intermediate type were seen in any of these rootlets. However, in view of the importance attributed to transitional types of pitting on tracheides by Seward (31), Penhallow (18), and Bailey (1), it should be mentioned, that tracheides with a transitional type of pitting were observed in some root-traces seen cut longitudinally in their course outwards through the secondary wood of the older roots to which some of these rootlets were found attached. Pitting of a transitional type was also seen on the tracheal walls in some of the 'parenchymatous' roots which are described later.

In well-preserved root sections no trace was found of the scalariform tracheides Osborn (17, p. 605) states to be present in the metaxylem of the primary wood of the rootlets of *Amyelon radicans*.

The phloem, in groups alternating in position with the xylem, consists of thin-walled elongated elements, many, but not all, of which are filled with black contents. These blackened elements, whose end walls appear to have been oblique, may have been sieve-tubes or possibly secretory cells. They show clearly both in the transverse and in the longitudinal sections, and are shown in the photographs reproduced in Pl. XXXIX, Figs. 1, 2, 4, and 10.

The anatomy of the rootlets is therefore sufficiently characteristic to identify them. The closely-packed multiseriate bordered pits on the walls of the tracheides of the metaxylem, the presence of elements with blackened contents in the phloem, and the nature of the cortex, undifferentiated except for the presence of fungal hyphae forming a 'fungal zone' round the stele, serve as diagnostic characters.

(iii) *The Formation of Secondary Tissues.*

The formation of secondary tissues occurred rarely, or at a late stage of growth in the majority of the rootlets, for out of the large number of sections examined, only seven were found which showed early stages of the development of secondary tissues. These seven rootlets varied in age, and successive stages in the development of the tissues were seen.

The secondary tissues developed in the same manner as they do in the roots of modern gymnosperms (de Bary (2, p. 553)). The cambium

was formed in the conjunctive cells lying between the phloem and the xylem plate, while the phellogen arose shortly afterwards in the outermost layer of the pericycle. In one root-section (B. Q 19, A 4) the cells lying between the phloem and the diarch xylem plate were seen to have divided, showing the initiation of the secondary vascular tissues, while the other cells of the root remained unchanged. In another section (C. A 283. 1) the radial rows of the periderm, about six cells deep, were seen on the inside of the endodermal cells and adjoining them, the radial rows of the peridermal cells alternating with the cells of the endodermis. A photograph of this section, reproduced in Pl. XXXIX, Fig. 8, shows clearly that the periderm originated in the layer of cells internal to the layer interpreted as the endodermis, that is in the outermost layer of the pericycle. It was concluded, therefore, that the phellogen arose in the pericambial layer in these rootlets, as it does in the roots of living conifers.

This point is stressed because the statements found in the literature concerning the place of origin of the periderm in the roots of *Amyelon radicans* do not agree. Osborn (17, p. 606) found grounds for thinking that the cork cambium was produced immediately outside the endodermis, while Scott (28, p. 287) and Seward (31, p. 87) give no exact place of origin of the periderm but state that the origin of the periderm in the British specimens was, doubtless, deep-seated. Renault (24, p. 295, Pl. XV, Figs. 13 and 17) who investigated the French specimens, figures it as occurring at the periphery of a layer composed of 'cellules polyédriques assez régulières', which is presumably part of the cortex.

Periderm formation in these rootlets would appear to have commenced shortly after the initiation of the formation of the secondary vascular tissue and to have taken place at the same rate during the early stages of secondary growth. In most of the rootlets in which early stages were seen, the radial rows of the periderm contained as many or more cells as those of the secondary xylem.

Shortly after the commencement of secondary thickening the cortex, including the 'fungal zone', was thrown off as a consequence of the deep-seated place of origin of the periderm.

(iv) *The Origin of Lateral Roots.*

The rootlets seemingly branched infrequently, for few cases of branching could be found. When branching occurred the lateral branch arose endogenously by the division of cells in the pericycle. The pericyclic origin of a lateral root is shown in the photograph of part of an obliquely transverse section of a rootlet reproduced in Pl. XXXIX, Fig. 9. In this section can be clearly seen the delicate-walled cells, many containing a nucleus, of the apical meristem of the young root. The new lateral root appears to have been formed by the division of cells on one side of the protoxylem

and not opposite to it as is the case in modern roots. More material, however, must be obtained before the exact place of origin can be determined.

It is perhaps worth while to put on record that a transverse section of one rootlet was found containing two small steles of equal size which were symmetrically placed near the centre of the section.

IV. THE ROOT APICES.

Very little is known concerning the structure of the growing points of the roots of fossil plants; this is not surprising as their tissues are delicate and their chance of preservation slight. Also the apices are so small that the plane in which they are sectioned must depend on chance.

It is therefore a matter of good fortune that in the slides available many sections of root apices should be present and cut longitudinally or in a plane at a slight angle to the longitudinal plane.

Altogether fifty-nine obliquely longitudinal or longitudinal sections of root apices were found, of which thirty-seven were oblique and twenty-two were, in part, longitudinal, though only some of these were cut approximately in the median plane. In addition to these fifty-nine sections, some transverse sections were noted which were in all probability sections of these root apices.

The majority of the sections seen were in the three nodules A, B, and C; ten were found in the preparations from nodule A, twenty-four in those from nodule B and eighteen in those from nodule C. The remaining seven were discovered in five disconnected preparations which each contained numerous other sections of the rootlets.

The state of preservation of the tissues of the root apices varies, in a few sections only is it so good that the structure of the most delicate of the tissues can be seen.

All that is known of the structure of the apices of the roots of British Coal Measure plants is to be found in the description by Osborn (17, p. 605) of one apex believed to belong to *Amyelon radicans*, and in that by Weiss (38) of two apices which he attributed to *Lyginodendron*.¹

Root apices have been recorded so rarely, and consequently so little is known of their structure, that it has been decided to choose the most nearly radial of the longitudinal sections and to describe each selected section separately. When this has been done not only will their anatomical structure be elucidated and their identity be established, but it is thought that sufficient data will have been given to support deductions which will be made concerning the mode of growth of the rootlets.

¹ One of these apices had been previously noted and figured by Stopes and Watson (33), p. 173 and Pl. XVII, photo 1.

Drawings, amplifying and illustrating the verbal descriptions, are reproduced in Text-figs. 1 and 2. They were all drawn from preparations made by cutting the nodules in the usual way, consequently the sections are more than one cell thick in the smaller-celled parts. In drawing these tissues, in certain cases a little difficulty was experienced when attempting to determine the exact order of the cell arrangement in any one plane, and this difficulty may have induced error. Another source of error is introduced by the possibility that some of the more delicate cell-walls may not have been preserved. Nevertheless each drawing is the result of an endeavour to represent the arrangement of the cells in one and the same plane as it appeared in an optical section of the root apex described. In the case of some of the root apices the drawings may be compared with the photographs reproduced in Pl. XXXIX.

(i) *The Structure of the Apices.*

Apex A. The section, figured in Text-fig. 1, A, was found in preparation Q 19, A 6 from nodule B and is considered to be a section through the growing point of a very young root apex in which the cells of the meristem were actively dividing and the tissues were still undifferentiated.

The section is slightly oblique, it passes through the stele at the apical end, then through the endodermis and finally through the cortex. The piece of root sectioned is very short, about 0.96 mm. long and lies practically free in the matrix of the nodule.

The identification of this apex rests on slender foundations. It was classed with the others because of the similarity of its general appearance to that of the other apices, and because it was found in nodule B which contains few other plant organs besides the roots and rootlets of this plant. In such a young apex as this is judged to be, the absence of the fully-differentiated characteristic cells of the stele is to be expected.

Apex B (Text-fig. 1, B). This apex terminates a short fragment of root, about 1.44 mm. long, found in nodule C (A 283. 1). Its tip is surrounded by vegetable debris. The root appears to have been bent just behind the apex and the tissues of the stele to have been partially destroyed.

The cortex with its characteristic 'fungal zone' and the presence in the stele of tracheides having multiseriate bordered pits on their walls serve to identify this rootlet.

Apex C (Text-fig. 1, C). The apex itself lies free in the matrix, but the rest of the root is crushed between other rootlets. The piece of root cut by the section is about 1.17 mm. long and was, judging from the section, the curved end of a rootlet. For the section, almost radial at the tip, passes obliquely through the stele, then through the plane of the endodermis and finally through the cortex and epidermis.

The presence of blackened elements in the phloem, the dark brown walls of the endodermal cells and the presence of fungal hyphae in an undifferentiated cortex determine the identity of this rootlet.

This apex was one of those found in nodule B (Q 19, A 3).

Apex D. This apex, also found in nodule B (Q 19, A 3), terminated a piece of root 1.75 mm. long. The tip is free in the matrix, but a very little further from the apex the rootlet is seen to lie between two other rootlets and to have its cortical tissue crushed on one side.

The section is cut almost in the median plane and the tissue-preservation is good, so all the characteristic features of these rootlets can be clearly seen. A multiseriately pitted tracheide in the metaxylem, blackened elements in the phloem, the dark-walled cells of the endodermis and the undifferentiated cortex can be seen in the drawing. The fungal zone of the cortex was seen in another part of the section. A photograph of the apex is reproduced (Pl. XXXIX, Fig. 10) as well as a drawing Text-fig. 1, D.

Apex E (Text-fig. 1, E). This was the first root apex seen and its discovery induced the close scrutiny of the slides which led to the finding of the other apices. The apex ends a detached piece of rootlet approximately 7.4 mm. long, which is cut longitudinally, but a little obliquely. The section is tangential to the stele at the extreme apex, then subsequently becomes radial and passes through the two protoxylem groups.

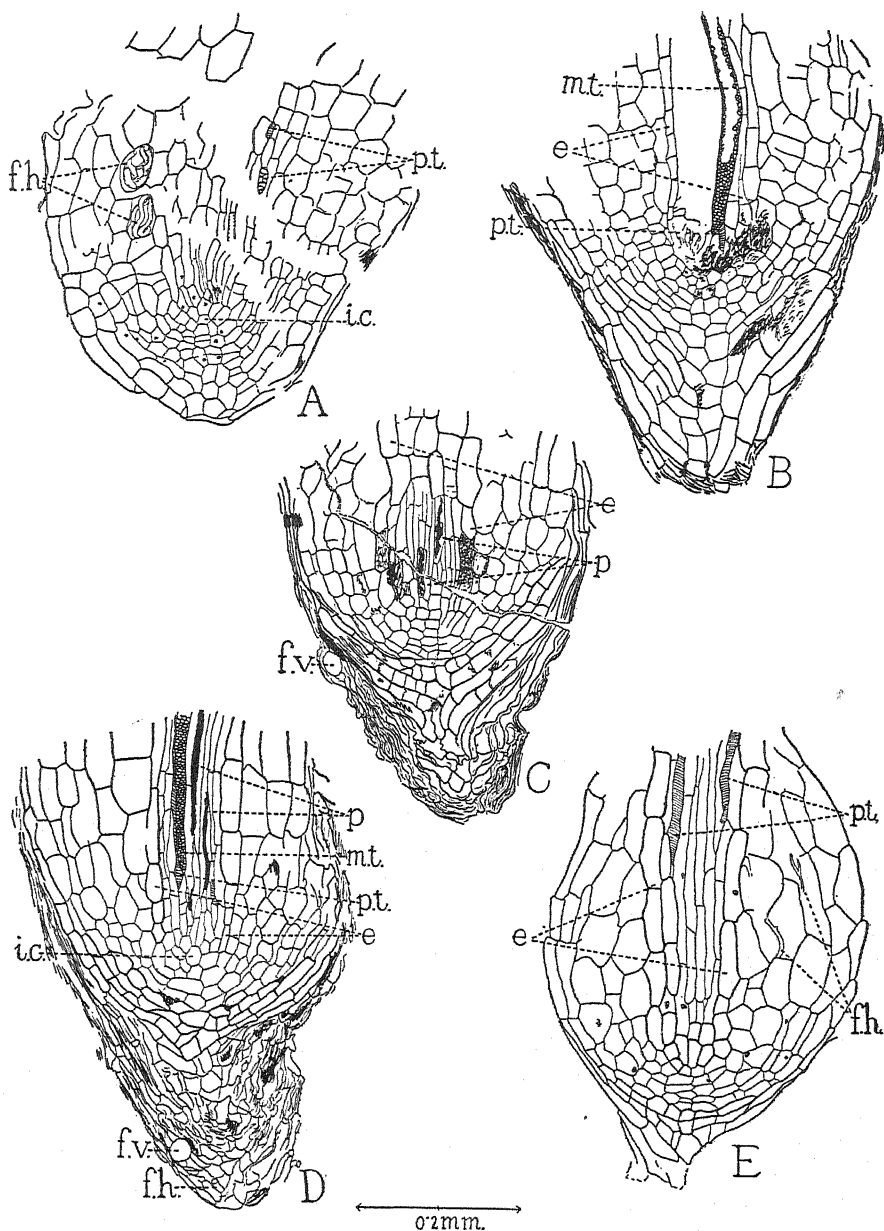
The apex itself lies free in the matrix and the preservation of its tissues is very good; not only can the form and arrangement of its cells be seen but structures resembling nuclei are present in some of the cells. A photograph of one of these cells with its nucleus is reproduced in Pl. XXXIX, Fig. 5. The tissue-preservation of the rest of the root is poor, for the root is crushed between other plant remains, nevertheless in places the structure of the stele can be seen.

This root is grouped with the others because the tracheides of the metaxylem have closely crowded bordered pits on their walls, and there are elongated cells with black contents in the phloem, a differentiated endodermis and a 'fungal zone' in the inner cortex.

The form and arrangement of the cells at the apex can be seen in the photograph, Pl. XXXIX, Fig. 6, as well as in the drawing (Text-fig. 1, E). The section is from nodule A, slide 52.

Apex F (Text-fig. 2, F). This apex was found in nodule B, slide Q 19, A 2, lying almost free in the matrix but in contact at two places with two other rootlets. The section was apparently cut through the short (0.93 mm.) bent end of a rootlet, for it appears to be cut radially at the extreme tip, then obliquely through the stele, endodermis and cortex successively.

The characteristic elements of the xylem were not present. The apex was identified through the similarity of its general appearance to that of



TEXT-FIG. 1. A-E. Drawings of the longitudinal sections of the root apices A-E respectively, showing the structure of the plerome and of the periblem including the root-cap. A description of the apices will be found in the text. *i.c.* = 'initial cell'; *e.* = endodermis; *pt.* = spiral tracheide of the protoxylem; *mt.* = tracheide of the metaxylem with walls covered with closely-packed rows of bordered pits in a poor state of preservation; *p.* = element of phloem with black cell-contents; *fh.* = fungal hyphae; *f.v.* = fungal 'vésicule'. Drawn with the aid of a Zeiss-Abbe camera lucida.

the other apices, the presence in the cortex of hyphae of the endophytic fungus and the brown-walled endodermal cells.

Apex G (Text-fig. 2, G). This apex is at the end of a short piece, 1.12 mm., of root. The section is cut a little obliquely, passing at the apical end through the stele and further down through the cortex. Except at the apex itself, the root is crushed between other plant remains and the preservation of the tissues is poor.

The root has fungal hyphae present in the inner cortical zone, elongated cells with oblique end-walls and black contents in the stele, presumably in the phloem, and the poorly preserved tracheides of the metaxylem show indications of having had crowded bordered pits on their walls. The possession of these characters and the general organization of the apex are sufficient to identify this rootlet. The section was found in preparation 53 from nodule A.

Apex H (Text-fig. 2, H). This apex also terminated a bent root, for the section goes through the median plane of the stele at the apex, while at a little distance from the apex it goes only through the cortex. The piece of root is about 1.36 mm. long and is partly surrounded by the poorly preserved tissues of another plant structure, apparently another rootlet.

This section showed all the characteristic features of these rootlets, the multiseriately pitted tracheides, the blackened phloem elements, the differentiated endodermis and the 'fungal zone' in the cortex. It was found in slide 25 in the Bedford College Collection.

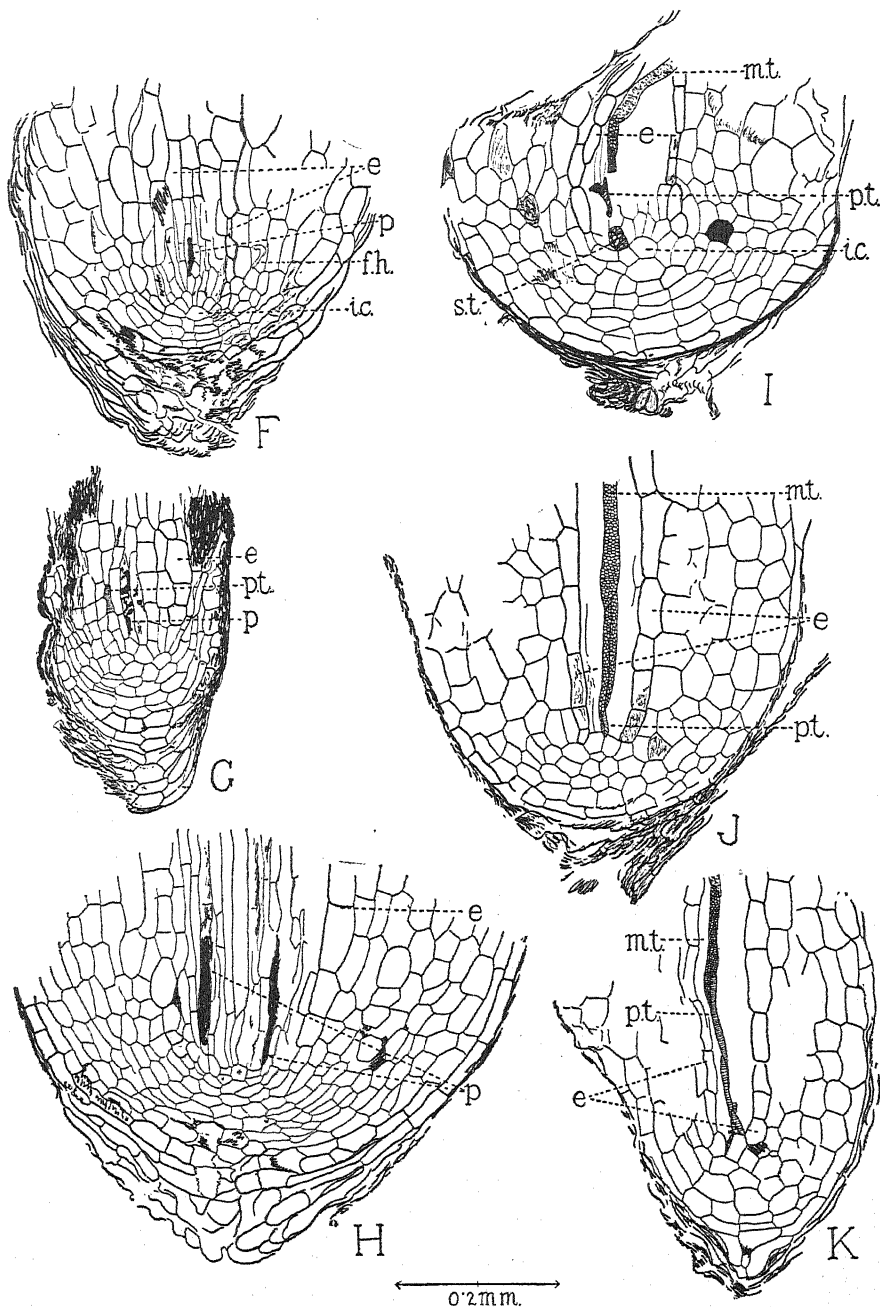
Apex I (Text-fig. 2, I). This apex ends a comparatively long, 3.95 mm., piece of rootlet. The extreme tip lies free in the matrix just touching a piece of plant debris at one side, but for the greater part of its length the rootlet lies crushed between other plant remains. The cells of the apex itself, except those at the top of the stele, are well preserved, the rest of the tissues were, however, destroyed.

Apex I is included with the others because of its general resemblance to them and the similarity in the structure of the cortex including that of the endodermis. Also the tracheides show indications of having had the typical multiseriate pitting on their walls, though the preservation is not sufficiently good to allow the type of pitting to be determined with certainty.

A short, broad tracheide is present in the stele apparently at the end of the vascular strand.

This apex was found in slide 26, in the Bedford College Collection.

Apex J (Text-fig. 2, J). This apex is another of the apices found in nodule C (A 283. 4). It terminates a piece of root about 4.33 mm. long which evidently was growing through a piece of parenchymatous tissue, for the tissues of the root are not crushed, though it is enclosed for most of its



TEXT-FIG. 2. F-K. Drawings of the longitudinal sections of the root apices F-K respectively, showing their structure, the differentiation of the stelar tissues to the apex of the plerome, the maturity of the cells of the periblem (J, K) and the remains of the root-caps. A further description of the apices is given in the text. *i.c.* = initial cell; *e.* = endodermis; *p.t.* = spiral tracheide of the protoxylem; *mt.* = tracheide of the metaxylem with walls covered with closely-packed rows of bordered pits in a poor state of preservation; *st.* = 'short' tracheide; *p.* = element of phloem with black cell-contents; *f.h.* = fungal hypha. Drawn with the aid of a Zeiss-Abbe camera lucida.

length by a piece of well-preserved tissue composed of somewhat thick-walled cells. The apical part, free in the matrix, projects beyond the enclosing parenchyma. The root evidently curved a little in growth, for the section, approximately median at the apex, passes successively through endodermis, stele, endodermis, stele, cortex, and stele before it ends when in the plane of the endodermis.

The section shows all the characteristic features of the roots, some of which are reproduced in the drawing J.

Apex K (Text-fig. 2, K). Apex K is another of the apices found in nodule C (A, 283. 7). It was found at the end of a piece of root, about 3.8 mm. in length, which was enclosed between two other rootlets with equally good tissue-preservation and lying approximately in the same plane. The apex itself appeared to be embedded in some decaying plant tissue. The rootlet evidently curved, the section is almost radial at the apex while further away from the apex it passes tangentially through the cortex.

The presence of tracheides with multiseriate bordered pits on their walls, the presence of fungal hyphae in the inner cortex, and the general type of organization of the apex are the grounds for the inclusion of this apex with the others.

The general organization of the growing point of the root can be determined from a comparison of the structure of the apices shown in the drawings. The apices differ in detail but have certain fundamental characters in common. The mode of growth of the root may be deduced from a study of these characteristic features which will therefore be considered in greater detail in the next section of the paper. Two salient features will, however, be pointed out here, the extension of the plerome nearly to the end of the root and the differentiation of the vascular tissues almost to the top of the stele.

The form of the root-cap, or, possibly its state of tissue-preservation, varies in the different apices. Its structure is probably seen best in apex B (Text-fig. 1, B). The walls of many of the cells of the root-caps are darker in colour and slightly thicker than those of the cortical cells and may have been suberized as was suggested by Osborn (17, p. 605) for those of a similar root-apex described by him and attributed to *Amyelon radicans*.¹

(ii) *The Mode of Growth.*

Our knowledge of the plants of Carboniferous times is of necessity somewhat fragmentary, and information that could be readily obtained from living plants is often entirely lacking. This is particularly true in

¹ The root apex described by Professor Osborn doubtless belonged to *Amyelon radicans*. Its characters appear to be the same as those of the apices now described.

regard to the methods of growth of the plants that lived in those days. The tissues of growing apices are very delicate and easily injured, so the chances of fortune are against their preservation. It is very fortunate, therefore, that in the preparations examined so many sections of root-apices are present and that the state of preservation in their tissues is so good. The details of the arrangement of the cells at the apex can be determined with sufficient accuracy to allow a conclusion to be reached as to the method of growth of the root.

The manner of growth of the roots of plants now living varies in the different groups of plants. There are two chief modes of growth; in the one growth follows from the division of a large single cell, the apical cell, in the other growth results from the divisions of a group of small cells, the apical meristem.

The drawings, reproduced in Text-figs. 1 and 2, of longitudinal sections of the apices of these fossil rootlets give details of the cell arrangement in the growing region. From a scrutiny of these drawings it can be deduced that growth took place as a result of the divisions of an apical meristem and did not follow from the divisions of a specialized apical cell. No indications of the presence of an apical cell were found in these or in any of the other sections examined, also the arrangement of the cells is not such as would result from the sub-division of segments cut off from a large apical cell, but is that which would follow from the successive divisions of a group of small cells. Even in the case of the young branch root, found at its place of origin in the pericycle, no sign of an apical cell was seen (Pl. XXXIX, Fig. 9). Growth in these rootlets must therefore have taken place through the activities of an apical meristem as it does in the roots of living phanerogams and lycopods.

Numerous investigators, whose work has recently been summarized by Schüepf (26), have contributed to our knowledge of the structure of root apices. The early workers soon found that the root apices of gymnosperms had a characteristic structure which differed from that of the majority of angiosperms.

Our knowledge of the apical meristems of gymnospermous roots is due to the work of Strasburger in 1872 (34), Reinke in 1872 (23), de Janczewski in 1874 (9), Flahault in 1878 (4), Schwendener in 1882 (27), and others whose results are all concordant. In 1912 Kroll (13), making a critical study of the structure of root apices, gives a list (13, p. 140) of the names of twenty-four gymnosperms, which includes representatives of all the modern groups, studied by various investigators. He states that the structures of the root apices in all these plants is the same in their general features.

De Janczewski's account of the root apex of *Taxus baccata* (9, pp. 189, and 190) might almost serve as a description of the apex of these fossil

rootlets. To make clear the close similarity, the main points of his description will be set forth and commented on. They are as follows:

(1) The root has at its apex only two independent, primary tissues, 'le cylindre central et l'écorce' (the plerome and periblem of Hanstein (7)). These two regions are present and are seen clearly in all the figures.

(2) The summit of the central cylinder is occupied by some short, large cells (the 'initial cells' of other writers) below which the cells of the cylinder begin to elongate. These 'initial cells' can be distinguished in some of the root-apices (A, D, F, and I), though not in all.

(3) 'L'écorce ne diminue nullement en épaisseur vers le sommet, où ses couches passent au-dessus du cylindre sans s'interrompre'. It can be seen that there is, as a rule, no decrease in the number of cell-layers of the periblem as it passes over the apex of the plerome, though the early maturity of the cortical cells results in a diminution in the size of the periblem at the top of the plerome.

(4) The exterior portion of this apical part of the cortex acts as the root-cap and its external layers are 'exfoliated'. The exfoliated layers are replaced by the divisions, more or less centripetal, which take place in the apical part of the cortex. This relationship between the apical part of the cortex and the root-cap can be seen in most of the drawings but is shown best by those in Text-fig. 1 in which the root-caps are well preserved.

(5) The 'epidermis' is composed of those portions of the cortical layers 'qui se montrent à la surface'. The connexion between epidermis, cortex and root-cap is best seen in the very young root apex, Text-fig. 1, A, though it is also shown in apices B, C, D, and G.

The close agreement of the apices with de Janczewski's description shows that the method of growth of these Cordaitean rootlets was, in every important point, the same as that of the roots of *Taxus baccata* and was therefore similar to that of all gymnosperms of modern days.

Nevertheless these Cordaitean rootlets exhibit some points of difference. The most striking of which are the small extent of the meristematic tissue and the early differentiation of the tissues. As a result of the small amount of the meristem the size of the periblem is also small. Modern gymnosperms differ in the amount of periblem present above the plerome. This was stated by de Bary (2, p. 13) in 1884 to be very small in *Cycas circinalis* and *Taxus*, but it would appear from Schwendener's figure (27, p. 247) to be less extensive in *Sequoia gigantea*, though even there the number of cell-layers between root-cap and plerome is greater than it is in these rootlets.

The other outstanding difference is the rapid differentiation of the various tissues and their resulting early maturity. Even in the very young root apex A, the cells of the cortex had attained their full size a few cells distant from the actively dividing apical group of cells, and tracheides of

the protoxylem with sculptured walls were found a short distance from the top of the plerome, while in the other apices (B, C, D, F, and G), in which the apical tissue shows signs of recent cell division, differentiation had proceeded further, and the tracheides of the metaxylem and the blackened elements of the phloem are differentiated almost to the top of the plerome. Also in some of the apices (C, D, E, F, and G) the walls of the endodermal cells show equally early differentiation, for they have the dark-brown colour of the secondary endodermis up to, but not enclosing, the summit of the plerome.

The smallness of the apical meristem and the early differentiation of the tissues may be correlated with limitation of growth in length of the rootlets.

This suggestion results from the number of apices found, three of which are figured in Text-fig. 2, I, J, and K, in which the cells of the apical tissue appear mature in form. The cells are larger and different in shape, as if they had come to maturity, and consequently the distance between the top of the plerome and the root-cap is greater. In those figured, I, J, and K, it is approximately twice (average = 0.111 mm.) as much as it is in the younger apices B, C, D, E, F, and H (average = 0.054 mm.).

Furthermore, the suggestion that growth is limited receives support from the fact that in certain of these roots special, short, broad tracheides were found at the termination of the vascular strand. These resemble 'storage' tracheides in form and are like those found in a similar position in the specialized tuberous roots of limited growth of *Ranunculus Ficaria* (Halket (6)). Drawings of these short tracheides are reproduced in Text-fig. 3, A and B.

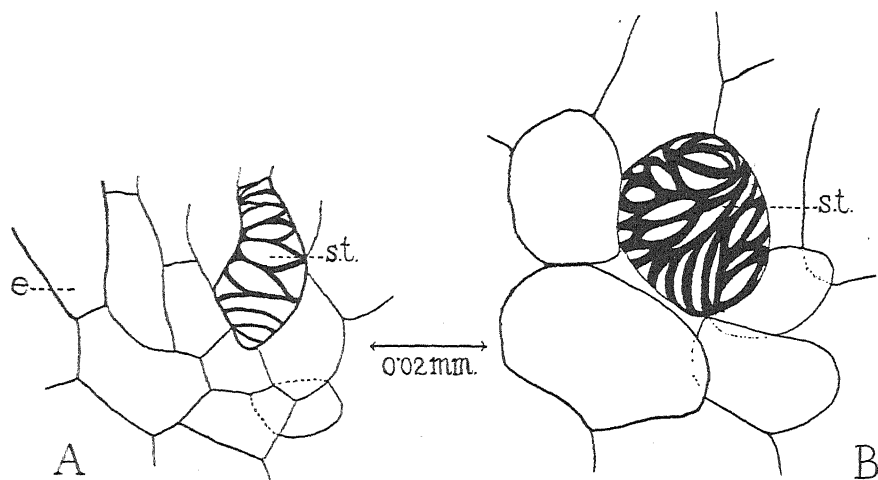
Additional evidence of the limitation of growth of these rootlets may perhaps be found in the differences seen in the structure of the root-cap.

In the case of fossil plants evidence based on the state of preservation of a tissue is rightly open to suspicion, but in these rootlets the state of tissue-preservation is, on the whole, so good that differences in the root-cap will be pointed out which could have resulted from the cessation of growth of the rootlets and are probably not due to the conditions of fossilization.

The form of the root-cap of a growing root is best shown in Text-fig. 1, B; the outer layers are darker in colour than the inner, more recently developed ones just above the summit. Successive later stages, when growth has ceased, are thought to be shown by C and D; E; K; and I and J. In C and D the inner layers are darker in colour and have probably become suberized, while the outer layers have become penetrated by fungal hyphae and have shrivelled up; in E these outer layers have disappeared as they have also in K, and in the mature apices I and J the whole of the root-cap has withered.

It would appear that a root-cap was present while the root was growing,

but when growth ceased no further cells were added to the cap which, consequently, became destroyed. It may be recalled that the root-cap behaves in a comparable way in roots of limited growth, as, for example, in the tuberous roots of *Ranunculus Ficaria*.



TEXT-FIG. 3. 'Short' tracheides found at end of xylem strands in some root-apices. A. One tracheide of group of three seen in an oblique section of a root apex found in slide Q 19. A 4, University College, London, Collection. B. Tracheide seen in root apex I, in slide 26 Bedford College Collection. *st.* = 'short' tracheide; *e.* = dark-walled cell of endodermis. Drawn with the aid of a Zeiss-Abbe camera lucida.

To sum up, it may be concluded that the method of growth of rootlets of this gymnosperm of Carboniferous times was the same as that of the roots of gymnosperms now living. At the growing apex only two regions, the periblem and the plerome, can be distinguished which formed respectively the cortex, including the epidermis, and the stele. The apical meristem was small in extent and consequently the plerome came to within a remarkably short distance of the root-cap. It is suggested that this comparatively few-celled meristem had, in the large majority of rootlets a restricted period of activity and that the growth of the rootlets was limited. Differentiation of tissues took place at an early stage, and when growth in length ceased the differentiation of the vascular tissues extended to the end of the stele.

V. THE ENDOPHYTIC FUNGUS.

The presence of the fungus was recorded by Osborn, who described its characteristic features as far as they could be determined in the material then available for examination. The tissue-preservation of the roots in nodules A and B is very good, the fungal hyphae are correspondingly well preserved, and consequently it has now been possible to determine more accurately the structure and distribution of the endophytic fungus.

(i) *Morphology and Distribution.*

Fungal hyphae are frequently present in the cortex of these rootlets, so frequently indeed that their presence can be regarded as one of the distinguishing characters of the cortex.

The mycelium, though found in the other parts, is present in greater quantity in the more central layers of the cortex. It is confined to the cortex and appears never to penetrate into the tissues of the stele. In one case only hyphae were detected in the phloem, and this was in a root whose tissues were obviously partially destroyed.

It is naturally impossible to be certain that only one fungus lived in the tissues, but, as most of the hyphae have the same morphological characters, the assumption is made that normally only a single fungus was present, though in some of the roots the occurrence of another fungus is possible. In root apex A, for instance, the nonseptate hyphae coiled in the cells (Text-fig. 1, A) very probably belonged to a second fungus.

No spores of any kind were found and the morphological characters of the vegetative hyphae are the only available criteria for the identification of the fungus. However, the vegetative characters are quite definite and serve to distinguish the fungus.

The fungal hyphae are septate. Transverse septa occur moderately frequently and are easily seen. They were apparently formed at irregular intervals as they are unevenly spaced. Septate hyphae can be seen in the photograph reproduced in Pl. XL, Fig. 17, in some of the other photographs, and in the drawings (Text-figs. 4 and 5). The hyphae branch frequently, but no relation between the position of the septa and the place of origin of the branches was noted.

The width of the hyphae is very variable and no reliance can be placed on the diameter of the hyphae as a distinguishing character.

The distribution of the hyphae is both intercellular and intracellular. In the outer cortex hyphae can be seen which appear to grow only between the cells, as is shown in drawings, Text-fig. 4, C and E, while other hyphae can be seen in the cells themselves. When present in the cells the hyphae are usually seen touching the cell-walls as if they grew in close contact with them.

In the inner cortex, in which, as has been pointed out, the bulk of the mycelium is found, the hyphae behave in a very characteristic fashion.

The fungal hyphae are best seen in longitudinal sections of the rootlets. In these the hyphae are seen to run longitudinally in the tissues and to grow most freely in the intercellular spaces (Pl. XL, Figs. 11 and 16), though they also occur within the cells.

The hyphae swell up into structures resembling the 'vésicules' of Janse (10, p. 63) so commonly present on the hyphae of the endophytic

fungi of mycorrhizae. The swellings here, however, apparently have in general thin walls and sparse contents.

The 'vésicules', or vesicles, vary in size and in form, as well as in position. They are in some cases terminal, in others intercalary. Formation of 'vésicules' appears to have taken place both in the intercellular spaces and in the cells themselves, and the position in which they developed evidently influenced their shape and size. In some cases they became quite large and approximately spherical, in others the form and dimensions seem to vary according to the space available for growth.

These vesicles may be the same structures as the 'large knob-like growths with thickened walls' described by Osborn (17, p. 607), and thought by him to be 'a form of resting body'. However, the walls of the vesicles do not appear to be especially thickened or the structures 'spore-like'.

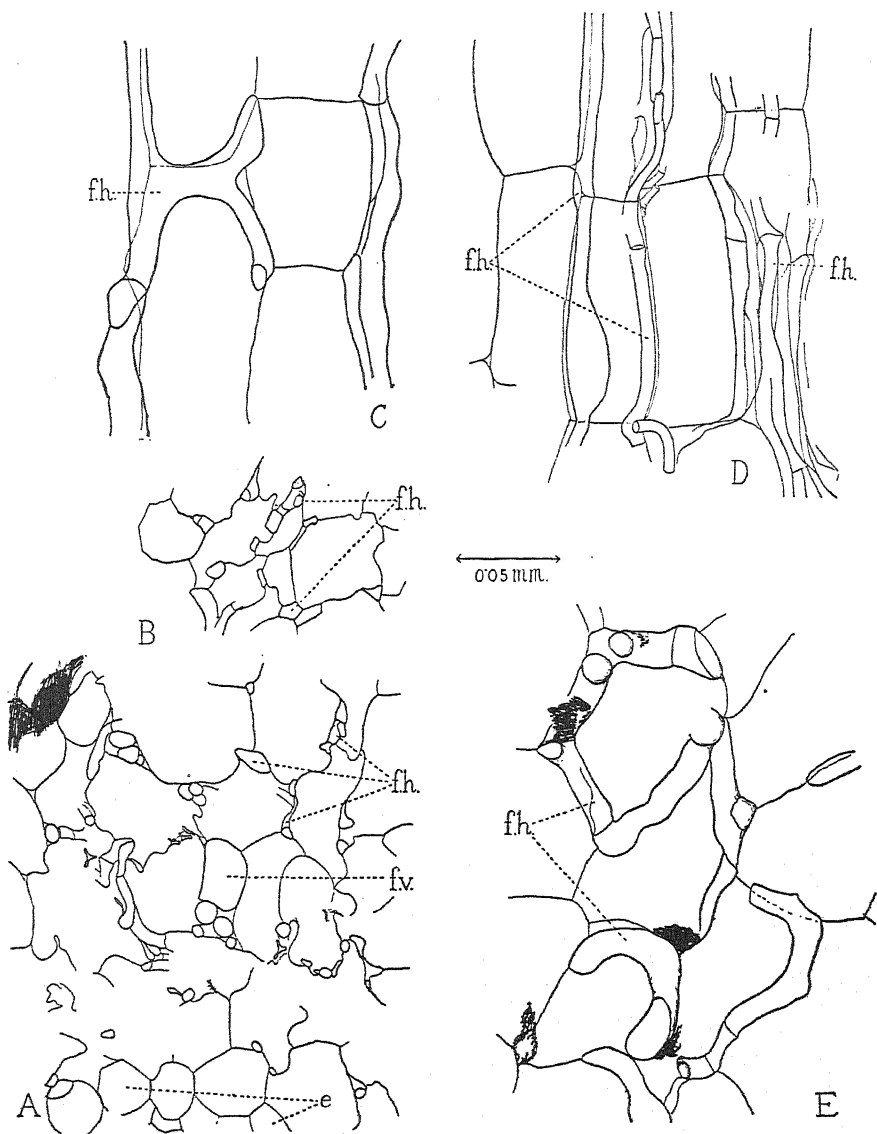
A photograph of fungal hyphae and 'vésicules' in the inner cortex, as they appeared in a tangential longitudinal section of a rootlet, is reproduced in Pl. XL, Fig. 18, while the general distribution of the mycelium as seen in a transverse section of the cortex of a young rootlet is given in the drawings, Text-fig. 4, A and B.

In most of the rootlets some of the cells of the inner cortex appear darker and fuller of contents than their neighbours, some of which, indeed, appear to be empty. Osborn recognized this, for he writes (17, p. 604), '... the cells here frequently have a dark cell contents. In some cases this occupies the whole cell, though in others it appears to have contracted towards the walls, or to form a more or less central mass with connecting strands to the walls. These masses of cell contents are apparently tangled knots of fungal hyphae filling the entire cell. . . . Some cells appear to be perfectly empty, though well preserved, as if the fungus had not affected them in any way'.

Osborn's interpretation of the exact nature of the dense cell contents may, I think, be amended as a result of the examination of better preserved material, though the association of the dark cell contents with the presence of the fungus is confirmed.

It was found that specialized branches were given off from the main hyphae running longitudinally in the intercellular spaces. One or more of these branches penetrated into the interior of a cell and there branched frequently, with the result that a compact cluster of branches was formed within the cell.

The cells being full of dark contents the branching of the fungal hyphae is difficult to trace, but it can be partially followed in a few instances. The branches arise close together and become successively smaller and smaller until the ultimate fine branches are lost in the general contents of the cell. The bushy clusters of branches so formed are similar



TEXT-FIG. 4. Distribution of fungus in cortex of rootlet. A. Transverse section of part of cortex of young rootlet, from slide 52, Bedford College Collection. B. Another part of cortex of same rootlet as A. C. Longitudinal section of part of outer cortex of rootlet showing wide septate hyphae in intercellular spaces. Slide 51, Bedford College Collection. D. Longitudinal section of another part of cortex of same rootlet as C, showing longitudinally running, branched, septate hyphae. E. Septate hyphae in intercellular spaces as seen in tangential longitudinal section of outer cortex of rootlet. Slide 55, Bedford College Collection. *e.* = endodermis; *f.h.* fungal hyphae; *f.v.* = fungal 'vesicle'.

to the 'arbuscules' found by Gallaud (5) and others to occur in the tissues of the endotrophic mycorrhizae of many modern plants and to be a characteristic feature of some endophytic mycorrhizal fungi.

In most cases the finer branches of the hyphae are difficult to see, and each invaded cell appears to be filled with a mass of heterogeneous substances giving it a dark and somewhat variegated appearance.



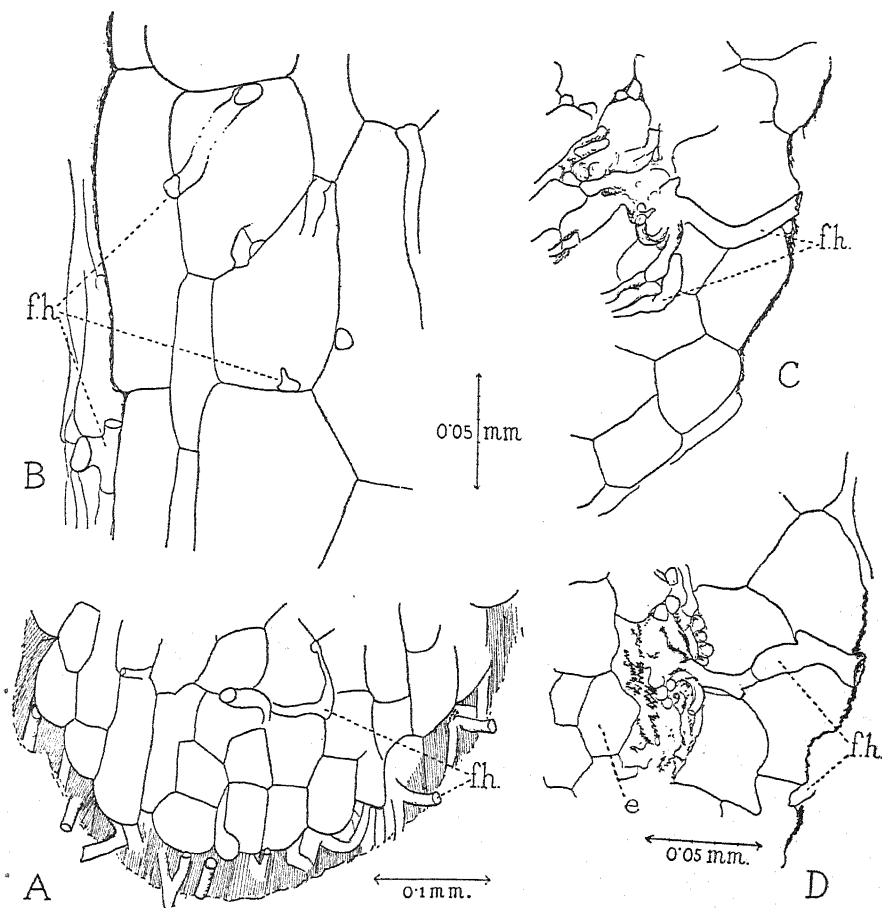
TEXT-FIG. 5. 'Arbuscules' in the cortical cells as seen in a longitudinal section of a rootlet. A. Part of the cortex with intercellular, septate hyphae and an intracellular branch cluster—an 'arbuscule'—in one of the cortical cells. B. Another cortical cell from the same rootlet showing much-branched, septate fungal hyphae, forming an 'arbuscule' in the cell. Slide 52, Bedford College Collection.

Photographs of some of these 'arbuscules' are shown in Pl. XL, Figs. 11-15, and drawings of two of them are reproduced in Text-fig. 5, A and B.

The fungus is almost entirely endophytic. Hyphae are sparsely present on the outside of the rootlet and are in most cases difficult to find. They run more or less longitudinally along the rootlet and the small transverse sections of the hyphae are consequently the only traces of the fungus found on the exterior of the roots when they are cut transversely. The hyphae can, however, be distinguished more clearly in oblique sections, and the drawing from one of these, reproduced in Text-fig. 6, A indicates their normal distribution on the surface of a rootlet. Two or three roots were, however, found which had a thin layer of hyphae on the

outside; a drawing of one of these, cut longitudinally, is reproduced in Text-fig. 6, B.

The mode of entry of the hyphae was difficult to determine. Search



TEXT-FIG. 6. The distribution of the fungus on the outside of the root and the mode of entry of the fungal hyphae. A. An oblique 'transverse' section of a rootlet with fungal hyphae on the epidermis. Slide 52, Bedford College Collection. B. A longitudinal section of the cortex with a thin layer of hyphae on the outside of the rootlet, and the intracellular hyphae in contact with the walls of the cortical cells. Slide 52, Bedford College Collection. C and D. Parts of a transverse section of a rootlet showing entering hyphae penetrating between the cells of the epidermis and branching in the inner cortex. Slide 52, Bedford College Collection. *f.h.* = fungal hyphae; *e.* = endodermis.

was made for entering hyphae, but they proved to be rare, and very few were found. The two which showed most clearly were discovered in a transverse section of a small root with a narrow cortex, and drawings of them are shown in Text-fig. 6, C and D.

The hyphae made their way between the epidermal cells, apparently

pushing them apart without injuring them. They seem to have grown perpendicularly to the surface in the direction of the inner cortex, and probably branched on the way. When the hyphae reached the inner cortex they behaved as already described and some of their branches penetrated into the cells and there branched repeatedly.

It can be seen therefore that the mycelium of the endophytic fungus had an interesting, restricted distribution and very distinctive morphological characters. The hyphae were septate and produced 'vésicules' and 'arbuscules' similar to those formed by many endophytic, mycorrhizal fungi of the present day.

(ii) *Biological Relations.*

It is interesting to speculate about the physiological relations that may have existed between the fungal hyphae and the tissues of the rootlet, notwithstanding the fact that the fruits of such speculation can never be put to the test of experiment.

Fungal hyphae are not infrequently found associated with the plant remains preserved in the calcareous nodules of the Coal Measures and in other rocks. Their presence is not surprising, rather would their absence from such a favourable habitat have required explanation.

One would expect that the majority of fossil fungi lived saprophytically on the decaying plant fragments undergoing petrification. Such, indeed, is the conclusion at which Kidston and Lang (11) arrived when investigating the abundant fungal remains present in the tissues of the plants of the Rhynie peat. They write (11, p. 872): 'It appears to be beyond doubt that many of the fungi were living as saprophytes and are associated with the decay of the tissues in which they are found.'

It is difficult to believe that the fungal hyphae present in the rootlets of *Amyelon radicans* were living there saprophytically. All the evidence deducible from their examination is against this conclusion. The chief facts unfavourable to the supposition are enumerated below.

1. The rootlets were apparently still growing when mineralization commenced, since the delicate tissues of some of the apices are preserved and the roots show the slight curvatures associated with growth.

2. The state of preservation of the tissues of the rootlet is equal to that of the mycelium of the fungus, and the cells show no signs of injury due to the action of the fungus.

3. The distribution of the fungal hyphae is localized, it is restricted to the cortex, and the greater part of the mycelium is to be found in the more central layers of the cortex. The fungal hyphae do not invade the stelar tissues.

4. Other plant tissues preserved in the same nodule do not appear to have fungal hyphae similarly associated with them, as might be expected

if the hyphae of a saprophytic fungus had been present ramifying through the partially decayed mass of plant fragments.

5. The constant association of the fungus with the rootlet.

It may reasonably be concluded therefore that the fungus was not, as a rule, living saprophytically on the tissues of a dead root. There are, however, no grounds for supposing that, if the infested root died the fungus did not go on living as a saprophyte on its dead tissues, for Peyronel (19) states that the endophytic mycorrhizal fungus of wheat, and of other plants, can continue to grow saprophytically in the roots of the plants after they are dead, as well as on other organic remains in the soil. Indeed, it is thought that the fungal hyphae with vesicles, found in certain of the root-caps of *A. radicans*, may have been the endophytic fungus living saprophytically on the decaying cells of the outer layers of the root-cap.

But, if the fungus were not normally a saprophyte, neither could it have been an injurious parasite. The intracellular clusters of branches—the ‘arbuscules’—might perhaps be compared to the haustorial, absorbing branches formed by some parasitic fungi, some species of *Peronospora*, but the state of tissue-preservation of the rootlet precludes the idea that the fungus was an injurious parasite living on and destroying the tissues of its host.

The good preservation of the tissues of the rootlet, the restricted distribution of the fungus, together with the fact that the fungus is found constantly present in the root, suggest rather that the fungus lived in a state of controlled parasitism or perhaps of ‘mutualistic symbiosis’ with the vascular plant. These facts suggest, indeed, that the relation between fungus and root was comparable to that existing between the two partners of a mycorrhiza. In fact the morphological characters of the fungus show such a close parallelism with those of the fungal partners of some endotrophic mycorrhizae that they may be considered to demonstrate that the physiological relations in the fossil root were similar to those existing in these endotrophic mycorrhizae of the present day.

The endotrophic mycorrhizae of modern plants may be divided into two groups, each distinguished by the morphological characters of the endophytic fungi (Rayner (22)).

The mycorrhizal fungi in one of these groups are characterized by the formation of specialized structures of two kinds, (1) the terminal or intercalary swellings of the hyphae—the ‘vésicules’—and (2) the intracellular dense clusters of branches—the ‘arbuscules’—including the structures formed by their degeneration, the ‘sporangides’ of so many writers. Endotrophic mycorrhizae of this type are of widespread occurrence,¹ and are formed by a large number of plants of varied affinities. They occur

¹ Gallaud (loc. cit.) and subsequent writers.

most frequently in herbaceous plants, but are formed by some conifers and other trees.

The rootlets of *A. radicans* have undeniable claims to be included in this group of endotrophic mycorrhizae since the morphology of the mycelium of its endophytic fungus is so similar. Its fungal hyphae are characterized by the formation of both specialized organs, they form 'vésicules' and 'arbuscules' similar to those of the fungi of the present day.

Furthermore, the main features of the distribution of the fungus in the tissues of the fossil root are similar to those found in endotrophic mycorrhizae of this group, though some differences occur. For instance, in the majority of modern mycorrhizae of this group the greater part of the mycelium is intracellular,¹ while in this fossil root it appears to be mainly intercellular though some of the hyphae, besides the 'arbuscules', are intracellular.

It should perhaps be mentioned here that the fungal symbiont in the fossil root may have belonged to a different class of the Fungi from that in which the fungi of the above modern mycorrhizae are placed. The identity of the endophytic fungus or fungi in endotrophic mycorrhizae of this type has not been established but, since their hyphae are characteristically non-septate, they are generally associated with the group of the Phycomycetes. The hyphae of the fossil fungus are septate and it would therefore naturally be placed in one of the other fungal groups, the Ascomycetes or the Basidiomycetes. However, the systematic position of the fungus will not be further considered now, as the identity of the fungus is of minor importance in a discussion of the physiological relationships existing between the fungus and the root.

The close similarity between the morphology and distribution of the fossil fungus and those of the fungal endophytes of some living endotrophic mycorrhizae has now been demonstrated. Kidston and Lang (11, p. 874), discussing the question of the occurrence of mycorrhizae in the Rhynie peat, write: 'Only convincing evidence from structural relations is admissible in a physiological question such as that under discussion.'

In the case of the rootlets of *A. radicans* such 'convincing evidence from structural relations' has been obtained, and the suggestion, first made by Osborn (17), that the rootlets of *A. radicans* were morphologically mycorrhizae may be regarded as substantiated.

(iii) *Systematic Position.*

Fungal hyphae are not infrequently found preserved in the Palaeozoic rocks, though very little is known of the systematic position of such fungi.

A summary of our knowledge of fossil fungi was given by Seward (31) in 1898, by Meschinelli (16) in 1902, and by Hirmer (8) in 1927, while

¹ Gallaud (loc. cit.) and subsequent writers.

recently our knowledge of the early Palaeozoic forms has been greatly increased as a result of the investigation, by Kidston and Lang (11), of the abundant fungal remains found in the Rhynie Chert Bed, Aberdeenshire.

The fungi from the Palaeozoic rocks apparently all have the same type of plant body, consisting of a typically non-septate mycelium, of vesicles, and of resting spores. Their structure shows general agreement with that of the modern phycomycetous fungi, and consequently these fossil fungi are usually classified with the Phycomycetes.

The fungus found in the rootlets of *A. radicans* differs from these fungi in at least two significant characters, the most important of which is the septate nature of its mycelium.

The presence of septa in the hyphae is apparently a constant character of the *Amyelon* fungus, and should cause the fungus to be separated from its phycomycetous contemporaries and classified with the higher fungi, if as much importance is attached to the formation of septa in fungal hyphae of Carboniferous times as is, as a rule, accorded to their presence in the hyphae of modern fungi.

The limitations imposed by the nature of the material become evident when considering such a subject as the importance to be attached to the septate character of the hyphae, for the mycelium can only be examined in one state, and variations due to nutritive conditions or to age cannot be determined. It should therefore be pointed out that, in spite of the fact that the fungal hyphae were septate in all the roots examined, the presence of the septa may possibly have been due to conditions of poor nutrition or to age, since septa are formed in these circumstances in the hyphae of certain phycomycetous fungi.

Nevertheless, the occurrence of typically septate hyphae in a fungus from the British Coal Measures is of particular interest, because records of the presence of similar fungi in the rocks of Palaeozoic age are extremely rare. In 1878 Cash and Hick (3, Pl. VI, Fig. 2C) figured a septate hypha; in 1895 Williamson and Scott, in an account of the primary structure of the root of *Lyginopteris* (*Kaloxylon hookeri*), mention that in the broad inner zone of the cortex 'branched and septate fungal hyphae are often met with' (40, p. 737); in 1912 Prankerd (21) recorded the presence of fungal hyphae in the soft parts of the seed of *Lagenostoma ovoides* and considered them as probably septate; while recently, in 1921, Kidston and Lang (11) describe, and give a photograph of, septate hyphae found in a partially decayed stem of *Asteroxylon*. The latter do not, however, consider that the mycelium of this fungus was typically septate. They write (11, p. 870), 'The one example in which transverse septa were fairly frequent and well marked appears exceptional among the numerous specimens examined, and this mycelium resembles in other respects the non-septate mycelium found in a corresponding position'.

The fungus of *A. radicans* may therefore be regarded as the first typically septate form to be described from the British Palaeozoic rocks.

The other distinguishing character which separates the *Amyelon* fungus from other known fungi of Palaeozoic times is the formation of the intracellular, dense branch-clusters called 'arbuscules', for, as far as has been ascertained, no similar structures have previously been described in fungi of that period. The possession of these structures, however, throws no light on the systematic position of the fungus.

No reproductive structures were found if one excepts the vesicles, which in modern fungi are regarded by Peyronel (19) and others as reproductive structures, sporangia, or spores. The vesicles of the fossil fungus so far examined have not the appearance of spores or of sporangia, and are, until further information is available, regarded as localized inflations of the vegetative hyphae.

In the absence of characteristic reproductive structures the allocation of the fungus to a precise systematic position is impossible. In the present state of our knowledge, the septate character of the mycelium might perhaps be considered to justify its separation from the Phycomycetes and its inclusion among the so-called higher fungi, but the available data do not allow a more exact classification.

In conclusion, it is of interest to recall here that though the modern endophytic mycorrhizal fungi, having 'vésicules' and 'arbuscules' and non-septate hyphae, are usually classed with the phycomycetous fungi, other suggestions for their classificatory position have been made. Gallaud, for example, laying stress on the chemical nature of their cell-walls, suggested an alliance with the Ascomycetes or the Basidiomycetes (5, p. 125), while Peyronel (19) thought the hypothesis should be considered that 'l'endophyte des mycorhizes et les *Endogone* tirent leur origine d'un type primitif, très variable de champignons (ou bien représentent éventuellement eux-mêmes ce type), duquel seraient dérivées deux séries divergentes de Phycomycètes et de Mycomycètes.'

VI. IDENTIFICATION OF THE ROOTLETS.

The identification of isolated rootlets is always difficult, and, in the case of fossil roots, the difficulties are multiplied by the limitations imposed by the nature of the material. Reliance must be placed on evidence obtained from anatomy, so in the case of the present rootlets the reasons for their identification with those of the Cordaitalean root, *A. radicans*, will be discussed in some detail, since their anatomical structure differs from that usually given for *A. radicans*.

The rootlets evidently grew somewhat massed together, since in the preparations numerous sections of the rootlets are found grouped together

in the matrix. The groups of sections were constantly found closely associated with sections of other roots of two types, older roots in which secondary tissues had been formed and roots characterized by the presence of a considerable amount of thin-walled parenchymatous tissue and comparatively little vascular tissue.

The frequent occurrence together of these three kinds of root structures suggested that they were probably all part of the same root system.

An examination of the sections proved this to be the case, since in a few instances a rootlet was found joined on to the tissue of an older root with secondary thickening, while in a greater number of cases rootlets were seen connected with the parenchymatous roots. Also the parenchymatous roots were seen attached to the older roots with secondary thickening.

The task of identifying the rootlet was simpler after it was found attached to an older root with a greater number of well-marked anatomical characteristics. A photograph of a triarch root with secondary tissues, showing the attachment of a rootlet is reproduced in Pl. XXXIX, Fig. 3.

Many sections of the older roots are present in the preparations, and their tissues are in some cases beautifully preserved, so that their anatomical structure can be examined in detail. A brief description of their anatomy is sufficient and is given below.

The primary xylem is generally triarch, but is sometimes diarch, tetrarch, or pentarch, and is surrounded by a zone of compact secondary xylem composed of tracheides and narrow medullary rays. The secondary wood is surrounded by a narrow band of phloem, then by a layer of parenchyma and a zone of periderm.

The secondary xylem is not uniform in appearance, narrow bands of cells with darker walls are present dividing the wood into layers and giving it the appearance of having annual rings. The walls of these dark cells are, however, not markedly thicker than those of their neighbours, though the cells are, on the average, slightly narrower radially. Neither is the wood always uniform in thickness on all sides of the root, apparently secondary thickening frequently took place irregularly since the 'layers' are of unequal thickness on the different sides, and are in some cases even crescent-shaped and do not extend round the root.

Many of the sections are cut a little obliquely, consequently the type of pitting on the walls of the tracheides can be seen.

The tracheides of the protoxylem are spirally thickened, those of the metaxylem, like the tracheides of the rootlets, have their walls covered with bordered pits. These pits are in closely-packed rows, the pits touching and alternating with each other so that their outer borders are irregularly hexagonal in outline. In some cases the elliptical outline of the pore can be seen, but in many instances the preservation is such that only the hexagonal outlines of the pits are visible on the tracheal walls. The

tracheides of the secondary wood have similar, closely-packed rows of bordered pits, which are, as a rule, found only on the radial walls.

The anatomy of these roots corresponds to the description generally given for the root of *A. radicans* except that the structure of the tracheides of the primary wood is different.

The tracheides of the metaxylem in *A. radicans* are, as a rule, stated to be scalariform, but are in these roots found to be pitted and to have many closely-packed bordered pits on their walls.

Our knowledge of the roots of *A. radicans* is due to the work of Williamson (39), who described the English specimens in 1874, and of Renault (24), who gave an account of the French forms in 1879. Later, in 1909, Osborn (17) contributed some additional interesting information.

In his description Williamson deals somewhat briefly with the structure of the primary wood. He does not mention the type of pitting on the tracheides of the primary xylem in the roots with secondary thickening, but he briefly describes (39, p. 70) and figures (39, Pl. IX, Fig. 53) a longitudinal section of part of a rootlet which passed through the central vascular strand. He says, 'The root-bundle consists of a few slender vessels, which are sometimes barred and at others reticulated'. The figure he gives adds nothing to this description. It seems probable that the 'barred' elements were the tracheides of the protoxylem, the 'reticulated' those of the metaxylem.

Comparison with the description and the figure (39, Pl. VIII, Fig. 51) given of the pitting on the tracheides of the secondary xylem shows that Williamson describes these too as 'reticulated' (39, pp. 68, 69). Furthermore, reference to the accounts he gives of other plants will show he described tracheides having typical multiseriate bordered pits as 'reticulated'. The term was doubtless due to a state of tissue-preservation in which only the hexagonal outlines of the pits were preserved, the outlines forming a net having irregularly hexagonal meshes, on the walls of the tracheides.

One may conclude, therefore, that Williamson used the term 'reticulated' to describe tracheides with multiseriate, closely-packed bordered pits when these were so badly preserved that only their outlines could be seen on the walls.

This interpretation of 'reticulated' tracheides, which was confirmed by the appearance of the tracheides in the roots in Williamson's preparations, removes the only objection to the identification of the root now described with *A. radicans*.

It was thought that reference to the work of Renault, who investigated the structure of the French specimens, might give additional information about the structure of the tracheides of the primary wood, but he gives no

description of these tracheides either in his original account (24) or later in his book (25).

However, as it has already been shown that close agreement exists with regard to their other characters, no further confirmation of the identity of the roots now described with the Cordaitalean root *A. radicans* is necessary, if this interpretation of Williamson's term 'reticulated' is accepted. Moreover the roots were compared with those in Professor Williamson's preparations (931 and 932) and were found to have the same anatomical structure as those he named *A. radicans*.

VII. COMPARISON WITH *RADICULITES RETICULATUS*, LIGN.

Attention should be drawn to the close resemblance between the rootlets of *Amyelon radicans* and those described by Lignier under the name of *Radiculites reticulatus*, though these come from the Stephanian rocks which are considered to be more recently formed than the Lancashire Coal Measures.

In 1906 Lignier (14) described some rootlets found in a single nodule in 'les silex stéphanien de Grand' Croix' near Saint-Étienne (Loire), and later in 1911, chance furnishing him with additional specimens, he was able to add to his observations, and to determine more definitely the affinities of his rootlets (15).

The drawings given of transverse sections of *R. reticulatus* indicate their resemblance to the rootlets of *A. radicans*, and the details given in Lignier's description further demonstrate their similarity. The anatomical features the rootlets have in common are, the undifferentiated cortex and the presence there of an endophytic fungus,¹ the appearance of the endodermis, and the structure of the stele.

In *R. reticulatus* the primary xylem is, as a rule, diarch though occasionally it is triarch; the walls of most of the tracheides of the metaxylem are 'couverts d'aréoles sur toutes leurs faces', the 'aréoles' being stated to be due to the hexagonal outlines of the badly preserved, closely packed pits.² The phloem contains elongated elements, with opaque red or black contents, some of which he regarded as sieve-tubes. Also no indications of the formation of secondary tissues were seen in most of the rootlets examined.

A single, but an important difference between *R. reticulatus* and the rootlets of *A. radicans* exists. In *R. reticulatus* the walls of the cortical

¹ Lignier suggests that the fungus was mycorrhizal. He figures part of the cortex 'dont certaines cellules sont envahies par des mycorrhizes'. He also pointed out that it was of interest that as early as the Stephanian epoch there existed 'l'envahissement des racelles par des mycorrhizes', loc. cit., Fig. 4 and p. 201.

² The tissue preservation is said to be poor, but Lignier thinks that, in the larger roots, tracheides with scalariform or a transitional type of pitting occur near the protoxylems.

cells have bars of thickening, except those of the cells of the two or three peripheral layers. The thickness of the bars was found to vary considerably, and was greatest on the walls of the cells adjacent to the endodermis. These bars of thickening on the walls of the individual cells, collectively form a network in the cortex, and are compared by Lignier to the thickenings, present on the walls of the cortical cells, in the roots of many living gymnosperms (Van Tieghem (35) and (36)).

These thickenings in *R. reticulatus* are described as 'un réseau lignifié', though it is obvious from the nature of the available material that the darker colour of the net must have been considered sufficient evidence of its lignified nature.

Lignier considered the presence of these thickenings a very important character; he writes (14 (1), p. 196), 'C'est ce réseau cortical qui m'a paru caractériser tout spécialement les organes étudiés...'. Consequently he distinguished this rootlet by the descriptive name *R. reticulatus*. At a later date he was able to establish the connexion between this rootlet and an older gymnospermous root with secondary tissues.

Lignier discusses at some length the question of the identity of the older root, comparing its structure with that of the root of *Poroxylon*, and with *A. radicans*. He decided that, though the root differed from both, it had more characters in common with *Poroxylon* than with *Amyelon*, consequently he suggested the identification of his roots with *Poroxylon*. However, as one of the characters Lignier considered important was the place of origin of the phellogen, it is possible that he would have decided otherwise, if it had been known then, as it now proved to be the case, that in *A. radicans* the phellogen was formed in the cells of the outermost layer of the pericycle as it was in *Poroxylon*, and in the root he was investigating. The only other difference of any importance he found between his root and *A. radicans*, was the absence in the secondary wood of *Amyelon* of 'rayons parenchymateux en face des pôles'.

Since the presence of 'un réseau lignifié' in the cortex is the outstanding difference between *R. reticulatus* and the rootlets of *A. radicans*, any information which enables the value of this character to be estimated more accurately is of interest.

For this reason it should be recorded that, among the numerous sections of roots examined, three transverse sections were noted, each in a preparation from a different nodule, which had dark thickenings on the walls of some of the cells of the inner cortex, more especially on the cells adjacent to the endodermis. Also dark bands, suggesting a net, were seen on the walls of the inner cortical cells in one oblique section.

These thickenings recall, and may be similar to Lignier's 'réseau lignifié', but they are more probably of the nature of 'artifacts', due to

the state of preservation of the fungal hyphae, since a similarity was observed between the thickenings on some of the cell-walls, and some badly preserved fungal hyphae present in certain of the adjacent cortical cells. The walls of the fungal hyphae were frequently darkened and sometimes appeared thickened, so that structures intermediate in appearance between hyphae and 'lignified bands' were seen.

The occurrence and the appearance of these bands of thickening on the cell-walls in some sections of the rootlets of *Amyelon*, suggest that possibly Lignier's 'réseau lignifié' may also have been due to the conditions of preservation, and have been fungal in origin, since he records the presence of an endophytic fungus in his roots. This suggestion receives a little support from the fact that Lignier found, in his preparations, one section of a root similar to the others, except that 'lignified' bands were absent from the walls of its cortical cells, an indication perhaps that the presence of the 'bands' was not a constant character.¹ If this were the case, the structure of the rootlets of *Amyelon* would be the same as that of *R. reticulatus*.

In consideration of the data now available, it is tentatively² suggested that the identification of *R. reticulatus* with the roots of *Poroxylon*, rather than with *A. radicans* should be reconsidered, though, when the close similarity existing between the roots of many modern conifers is remembered, it is doubtful whether the assignment of *R. reticulatus* to a more precise systematic position than that of the root of a Cordaitalean plant can be justified.

VIII. THE ROOT SYSTEM OF *AMYELON RADICANS*.

Williamson (39), in 1874, indicated that the root system of *A. radicans* was a complex one. He speaks of, and illustrates (Pl. VII, Fig. 46) 'clusters of diverging rootlets which stream in every direction'. He also gives a figure (Pl. VIII, Fig. 48) of 'one of these root-clusters commencing as a large globular mass dividing into separate rootlets'. Williamson thought that these clusters of rootlets were arranged irregularly on the periphery of the bark. Osborn (17) also found that 'tufts of short much-branched roots were borne at intervals on *A. radicans*'.

New facts increasing our knowledge of the origin and structure of these clusters can be added as a result of the present work.

It may be remembered that sections of three root structures, namely: older roots with secondary thickening, 'parenchymatous' roots and rootlets,

¹ Lignier considered this difference so important that he thought this rootlet belonged to another plant.

² This suggestion is made very tentatively since no examination of Professor Lignier's preparations has been made.

were constantly found associated, and also that these were all connected together. Rootlets were found attached both to the roots with secondary thickening, and to the 'parenchymatous' roots, while the latter were found joined on to the older roots.

The parenchymatous roots appear to have been specialized structures quite different anatomically from the other roots. They arose endogenously from the normal roots, and when young had root-caps, but the sections seen were insufficient for the determination of the details of their mode of origin. The photograph reproduced in Pl. XXXIX, Fig. 3 shows two of these parenchymatous roots attached to the 'cortical' tissues of an older root. One of these has a root-cap and is still enclosed in the outer tissues of its parent root, but the junction of the root-trace with the vascular strand of the parent root cannot be seen. The second root appears to be slightly older than the first. The apparent maturity of the tissues in these two small parenchymatous roots suggests that they grew slowly.

The parenchymatous roots branched frequently, the branches often not separating from each other, so that irregularly shaped masses of parenchymatous tissue were formed. These are the structures Williamson (39) figured in Pl. VII, Fig. 46, and in Pl. VIII, Fig. 48, while Osborn (17) reproduced a photograph of a similar branch cluster in Pl. XLVI, Fig. 1.

An examination of these irregularly shaped structures showed that much of the parenchymatous tissue of which they were composed was formed by secondary growth, but the manner in which this was effected was not determined.

Moreover, the vascular strands in the parenchymatous roots differed from those in the other roots, many of the tracheides were shorter and broader, resembling 'storage' tracheides in form and, as mentioned earlier, some at least of the tracheides had a transitional type of pitting on their walls.

It should be noted also that no 'fungal zone' was developed in the 'parenchymatous' roots.

In a number of instances rootlets were seen attached to the parenchymatous roots, and it was probably on these roots that the majority of the rootlets were borne, though rootlets were also attached directly to the normal roots, as is shown in the photograph on Pl. XXXIX, Fig. 3.

A minority only of the rootlets developed a cambium, forming secondary tissues, and it is thought that these may perhaps have been the rootlets which arose directly on the branches of the main root system.

The root system of *A. radicans*, therefore, in all probability consisted of a main root bearing normal lateral branches which continued growing, forming secondary tissues and branching in the normal way; but having in addition specialized short, frequently branched, somewhat tuberous roots, which developed little secondary wood. The parenchymatous

roots bore many rootlets, which were probably rootlets of limited growth, whose apical meristem after functioning for a short period ceased to grow, and in which a cambium did not develop.

It would appear that *A. radicans* had, in short, a branching root system of the normal type, on which were formed specialized, somewhat tuberous roots bearing clusters of rootlets.

The rootlets are very interesting and specialized structures. Many, apparently, were of 'limited growth', and all had an endophytic fungus present in the cortex.

The mycorrhizal nature of these rootlets has already been discussed, and it has been shown that, as far as could be determined from their morphological characters, the relations between fungus and root were the same in this palaeozoic gymnosperm as in the endotrophic mycorrhizae of *Sequoia gigantea* (5) and other conifers, and in many other modern plants. The occurrence of this mycorrhiza in the carboniferous era has the additional interest of being the first fossil 'mycorrhiza' of this type to be described. *Mycorhizonium*, the first 'mycorrhiza' to be described from the British Coal Measures (Weiss (38)) was also an endotrophic mycorrhiza, but it apparently belonged to the other group of endotrophic mycorrhizae for 'characteristic clump formation' was observed in the cells (Verdauungszellen) of the medio-cortex.

The *Amyelon* rootlets, moreover, may have been specialized organs in respect to their water-absorbing capacity, though naturally very little evidence can be brought forward in support of this suggestion.

The conclusion has recently been drawn by Popesco (20), by Scott and Priestley (29), and by Scott (30) that the absorbing region of the root lies between the zone in which the membranes (endodermis and exodermis) are completely suberized and the apical meristem.¹ It is of interest, therefore, to point out that in the mature rootlets of *Amyelon*, the differentiation of the endodermis extends to the top of the plerome, and to a very short distance (average = 0.06 mm.) from the base of the root-cap. Consequently, if the walls of the differentiated endodermal cells were suberized, as appears probable from their darker colour and more resistant nature, the absorbing zone of the rootlet would be extremely small, and its water-absorbing capacity would be correspondingly reduced. It is possible, therefore, that these mycorrhizae of limited growth had very little water-absorbing capacity.

Our knowledge concerning the habitats of carboniferous plants is very limited. These roots of *A. radicans*, apparently grew in partially decayed vegetable debris, the rootlets curving slightly in growth and occasionally

¹ Popesco considers that the level at which the differentiation of the vessels begins is the lower limit of the absorbing zone.

penetrating the surrounding plant fragments, which were frequently in an advanced condition of decay. The fact that many of the apices are found free in the matrix, indicates that this debris had large spaces between the decaying plant fragments into which the rootlets grew.

These spaces may have been full of air, or of water. It would seem more probable from the anatomical structure of the rootlets that the former was the case, and that the root grew in humus, rather than in a swamp or marsh as suggested by Osborn (17, p. 610).

Furthermore, the good preservation of the tissues, especially of such delicate structures as root apices, supports the suggestion that the plants were still growing when petrification took place (17, p. 609), also (33).

Roots are rightly regarded as conservative organs, and the measure of the conservatism of the gymnospermous root is revealed by the following comparison: In the palaeozoic gymnospermous root *A. radicans*, the anatomical structure, the place of origin of the phellogen, the origin of lateral branches, the structure of the growing apex and even the biological relations with an endophytic fungus were all the same as they are in certain conifers of the present day.

IX. SUMMARY.

1. The rootlets of *A. radicans* had a characteristic anatomical structure. The distinguishing features were as follows:

(a) The cortex was not differentiated into distinct regions, but the inner cortex was characteristically inhabited by fungal hyphae, so that a 'fungal zone' was present round the stele.

(b) A differentiated endodermis was present. In some very young roots the cells had the characteristic 'Casparian strip' on their walls, but in the majority of the roots examined the cell-walls were uniformly dark brown and were, in all probability, suberized.

(c) The rootlets were, as a rule, diarch. The tracheides of the metaxylem had their walls covered with closely-packed rows of bordered pits.

(d) Elongated elements, full of black contents, were present in the phloem. These were probably sieve-tubes, but may have been secretory cells.

(e) Root hairs were not, as a rule, developed.

2. The majority of the rootlets, apparently, did not form secondary tissues, but in those in which these tissues were formed the cambium and the phellogen developed approximately at the same time. The phellogen originated in the stele, in the pericambial layer.

3. The rootlets rarely branched, but when lateral branches were formed they arose by the division of cells in the pericycle. The exact place of origin, in relation to the protoxylem groups, could not be determined.

4. Many root apices were found and their structure is described. Their general organization was the same as that of the root apices of modern gymnosperms, but the meristem was very small and the tissues were differentiated at an early stage. The structure of the apices shows that the rootlets grew by the division of a group of cells, and differences in the structure are pointed out which indicate that many of the rootlets had 'limited growth'.

5. The hyphae of the fungus present in the inner region of the cortex were septate and were, apparently, mainly intercellular but were also partly intracellular. Structures were formed comparable to the 'vésicules' and 'arbuscules' characteristic of the endophytic fungi of certain modern mycorrhizae.

6. The physiological relations between fungus and root are considered; they are regarded as having been of the same nature as those that exist between the fungus and the root in certain endotrophic mycorrhizae of the present day.

7. The septate mycelium indicates that the fungus may have belonged to the Ascomycetes or to the Basidiomycetes, rather than to the Phycomycetes.

8. Reasons are given for the identification of the rootlet with *A. radicans*, Will.

9. The rootlets closely resemble those described by Lignier under the name of *Radiculites reticulatus*, and it is suggested that *A. radicans* and *R. reticulatus* are the roots of the same plant or of nearly allied plants.

10. The root system of *A. radicans* apparently consisted of a normal branching root system, bearing on its branches short, somewhat parenchymatous roots which branched frequently, and which bore clusters of mycorrhizae of limited growth.

11. The palaeozoic gymnospermous rootlets of *A. radicans*, belonging in all probability to *Cordaites*, were found to be similar to the roots of many gymnosperms of the present day in anatomical structure, place of origin of the phellogen, origin of lateral branches, structure of root apex, mode of growth, and to have had physiological relations with an endophytic fungus similar to those existing in the mycorrhizae of certain modern conifers and other plants.

In conclusion, I have pleasure in gratefully acknowledging my indebtedness to Professor F. W. Oliver, who lent me several series of beautiful preparations. My thanks are also due to Professor D. M. Watson for

permission to make use of certain of his preparations. I am also indebted to the Geological Department, British Museum (Natural History) for facilities to examine preparations in the Williamson Collection.

BEDFORD COLLEGE,
UNIVERSITY OF LONDON,
June, 1929.

ADDENDUM.

Since the above was written I have found in M. Alfred Carpentier's paper 'Sur les végétaux à structure conservée d'un silex permien' (Rev. Gén. de Botanique, tom. xxxvi, 1924) a short account of some 'Racines de Cordaitales' (pp. 246-249).

It should be noted that M. Carpentier considers his rootlets similar to Lignier's *Radiculites reticulatus*, and states that they also resemble the rootlets of *Amyelon radicans*, Will., described and figured by Osborn (17).

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Halket.—The Rootlets of 'Amyelon radicans', Will.;

EXPLANATION OF PLATES XXXIX AND XL.

Miss A. C. Halket's paper on the Rootlets of *Amyelon radicans*, their Anatomy, their Apices, and their Endophytic Fungus.

Bedford College Collection. U.C.L. = University College, London, Collection.

W. = Professor Watson's Collection.

PLATE XXXIX, Figs. 1-10 (Photographs).

A transverse section of a rootlet showing the relative proportions of stele and cortex. One (*f.z.*) of the inner cortex, the endodermis (*e.*), the diarch xylem (*x.*) and the elements of the phloem (*p.*) can be seen. B.C. 52 (Shore). $\times 78$.

Part of a longitudinal section of a rootlet showing the relative proportions of stele and the inner region of the cortex the fungal zone with longitudinally running hyphae (*f.h.*), (*f.v.*) and 'arbuscules' (*a.*) can be seen. The endodermis (*e.*) is clearly shown, also the pitted tracheides (*m.t.*) of the metaxylem and the blackened elements (*p.*) of the phloem. B.C. 52 (Shore). $\times 71.4$.

This photograph shows the connexion of a rootlet (*r.*) and two young 'parenchymatous' roots (*p.r.*) with the periderm (*pd.*) of an older root (*o.r.*) of *Amyelon radicans*. The origin of the younger parenchymatous root is shown and its root-cap (*r.c.*). Sections of the older root can also be seen. B.C. 52 (Shore). $\times 20$.

A longitudinal section of part of the stele of a rootlet showing a tracheide of the metaxylem with its wall covered with closely-packed bordered pits. The elliptical openings of the tracheide are clearly seen. *p.* = blackened element of the phloem. B.C. 52 (Shore). $\times 350$.

A cortical cell, containing a structure presumably a nucleus (*n.*), from the longitudinal section of root apex E (see Text-fig. 1, E and photograph, Plate XXXIX, Fig. 6). B.C. 52 (Shore). $\times 50$ approximately.

A longitudinal section of root apex E. *r.c.* = root-cap; *e.* = endodermis. See p. 874, and the text, Text-fig. 1, E. B.C. 52 (Shore). $\times 100$.

A longitudinal section of root apex G. *r.c.* = root-cap; *e.* = endodermis; *p.t.* = proto-blackened elements of phloem. See p. 876, also drawing in the text, Text-fig. 2, G. $\times 200$.

A transverse section of a diarch rootlet preserved shortly after the commencement of thickening. The radial rows of the peridermal cells (*pd.*) can be seen within and concentric endodermal cells (*e.*), indicating that the phellogen originated in the pericambial cortex (*c.*) is still present. W. A 283, 1 (Shore or Oldham). $\times 50$.

A obliquely transverse section of a diarch rootlet showing the endogenous origin of a branch (*b.r.*), which is still within the endodermis (*e.*), apparently arose on one xylem group (*x.*). The delicate walls of the cells of the young rootlet are well preserved, in all probability, nuclei (*n.*), can be seen in the cells. *p.* = phloem with blackened contents, *c.* = cortex. B.C. 31 (Shore). $\times 125$.

A longitudinal section of root apex D, *r.c.* = root-cap which has fungal hyphae and its outer layers; *e.* = endodermis; *p.t.* = protoxylem tracheide; *p.* = blackened elements of phloem. See p. 874, also drawing in Text-fig. 1, D. U.C.L. Q 19, A 3. $\times 100$.

PLATE XL. Figs. 11-18 (Photographs).

Part of cortex of section shown in Plate XXXIX, Fig. 2 enlarged to show more longitudinally running, intercellular hyphae (*f.h.*) and two 'arbuscules' (*a₁* and *a₂*). The pitted tracheides (*m.t.*) and the blackened elements (*p.*) of the phloem can also be seen. B.C. 52 (Shore). $\times 200$.

Fig. 12. Part of Fig. 11, enlarged to show the septate hyphae (*f.h.*) and one of the 'arbuscules' (*a*₁). A drawing showing the hyphae and the 'arbuscule' is reproduced in the Text-fig. 5, A. B.C. 52 (Shore). $\times 460$ approximately.

Fig. 13. Some cortical cells from another part of the same rootlet as Fig. 11, showing an 'arbuscule' (*a*) in one of the cells. A drawing showing the branching of the hyphae, which form the 'arbuscule' in the cell, is reproduced in the Text-fig. 5, B. *e.* = endodermis. B.C. 52 (Shore). $\times 345$ approximately.

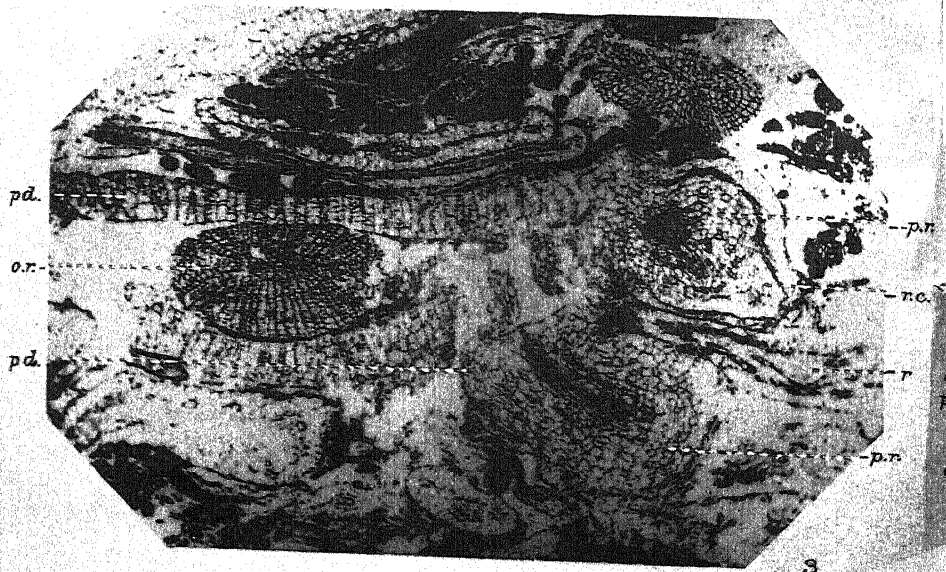
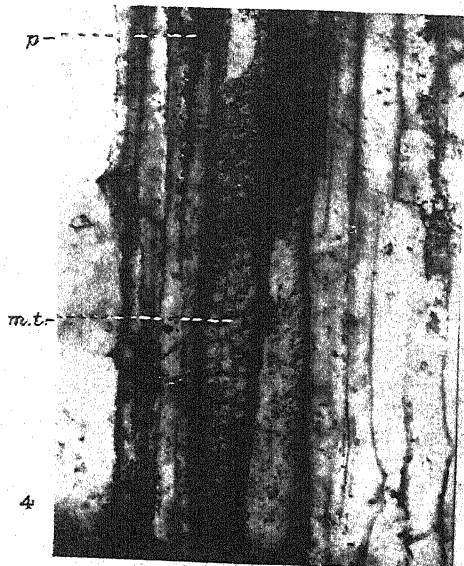
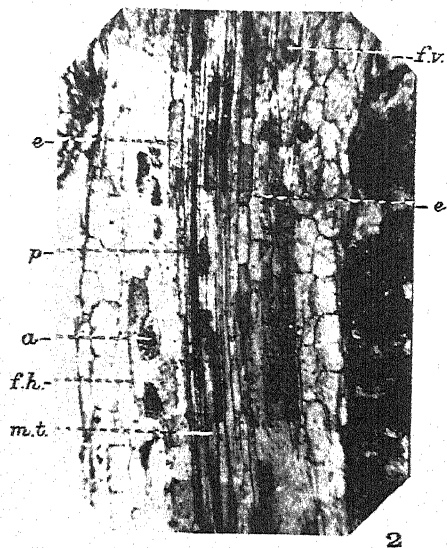
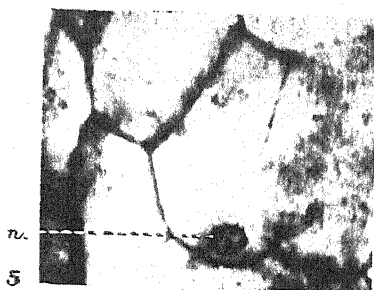
Fig. 14. Part of a longitudinal section of the cortex of a rootlet showing the longitudinally running, intercellular hyphae (*f.h.*) and three 'arbuscules' (*a*) in three of the cells. B.C. 51 (Shore). $\times 325$ approximately.

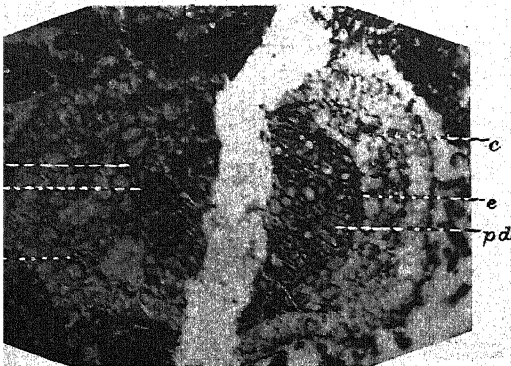
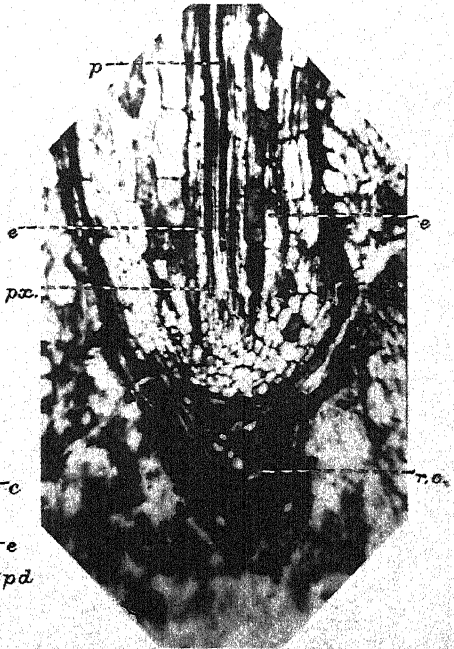
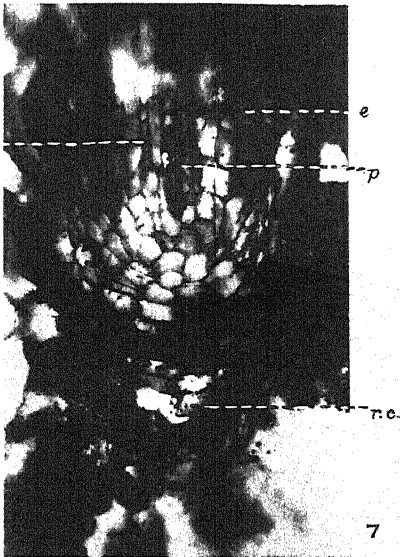
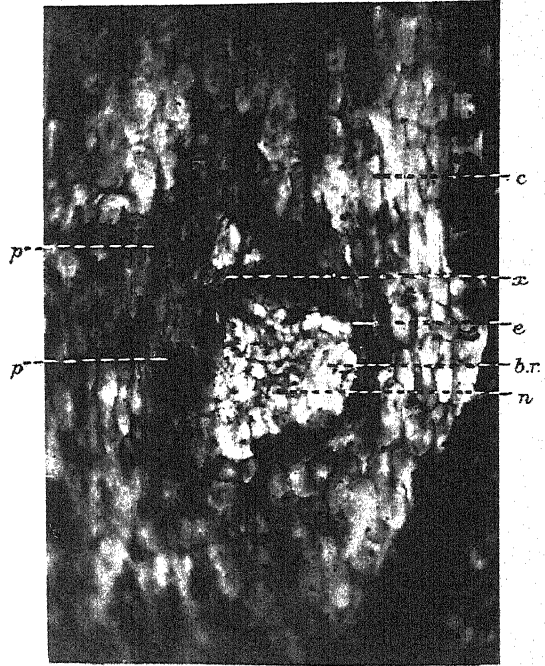
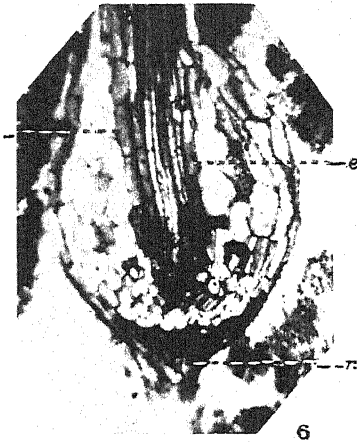
Fig. 15. Part of a longitudinal section (slightly oblique) of a rootlet showing 'arbuscules' (*a*) in some of the cells of the inner cortex. *e.* = endodermis. B.C. 52 (Shore). $\times 200$.

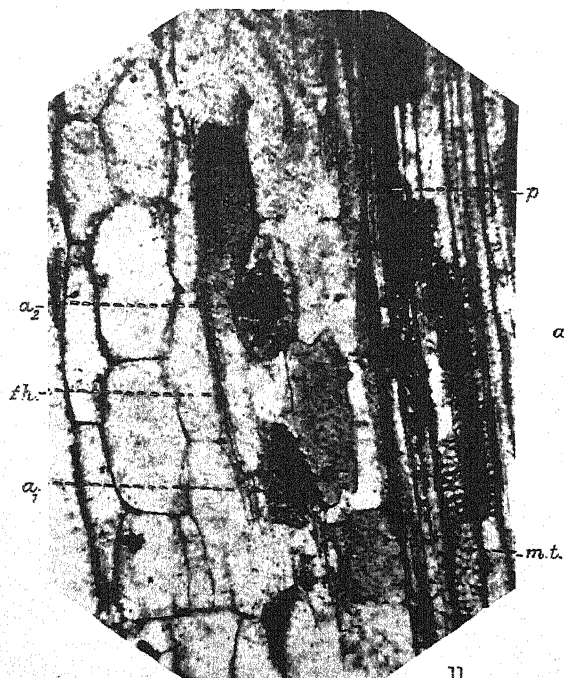
Fig. 16. Part of a longitudinal section of the cortex of a rootlet showing the longitudinally running hyphae. The hyphae (*f.h.*) apparently grew in the intercellular spaces. Cross septa can be seen in some of the hyphae and a swelling (*f.v.*) (a young 'vésicule'?) in one of them. A drawing of part of this section is reproduced in the Text-fig. 4, D. B.C. 51 (Shore). $\times 260$ approximately.

Fig. 17. Fungal hyphae (*f.h.*) from the cortex of a longitudinal section of a rootlet. The cross walls (*s*) in the hyphae are clearly shown and an irregularly shaped 'vésicule' (*f.v.*) is also present. B.C. 51 (Shore). $\times 300$.

Fig. 18. Tangential longitudinal section of the cortex of a rootlet, showing the fungus in the inner region of the cortex (*f.s.*). Cross septa can be seen in many of the hyphae. A terminal 'vésicule' (*f.v.*) can also be seen, as well as other 'vésicules'. B.C. (Shore). $\times 200$.



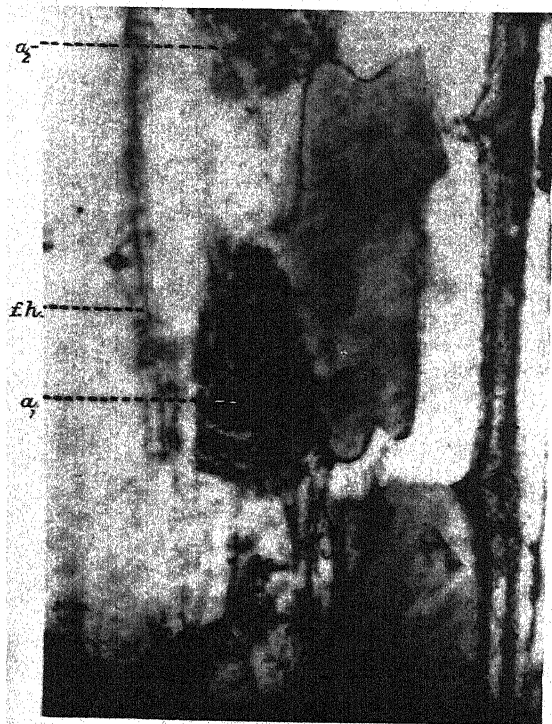




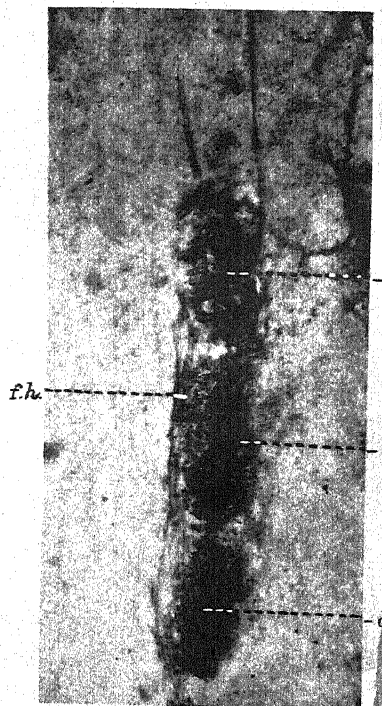
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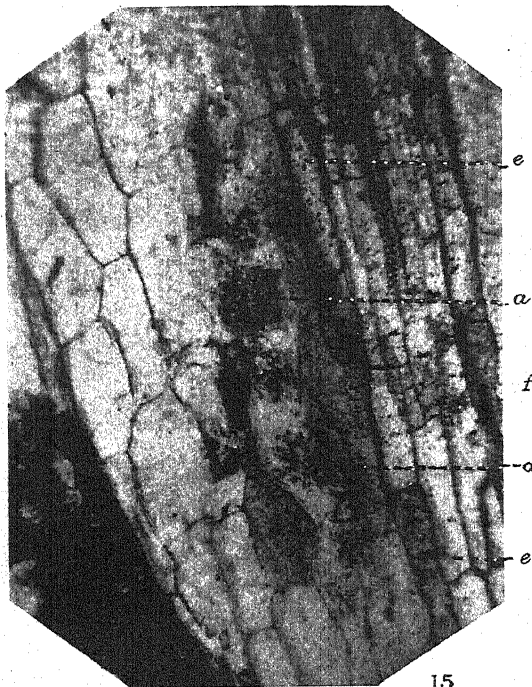
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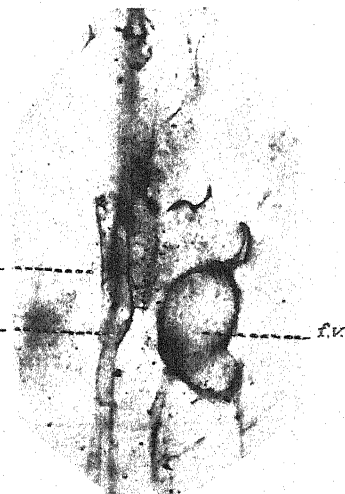
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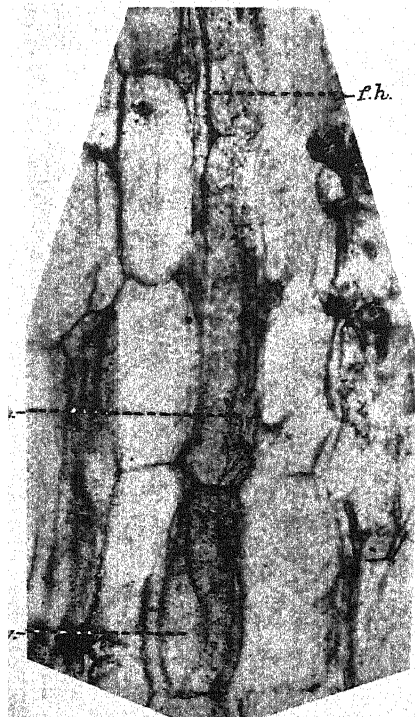
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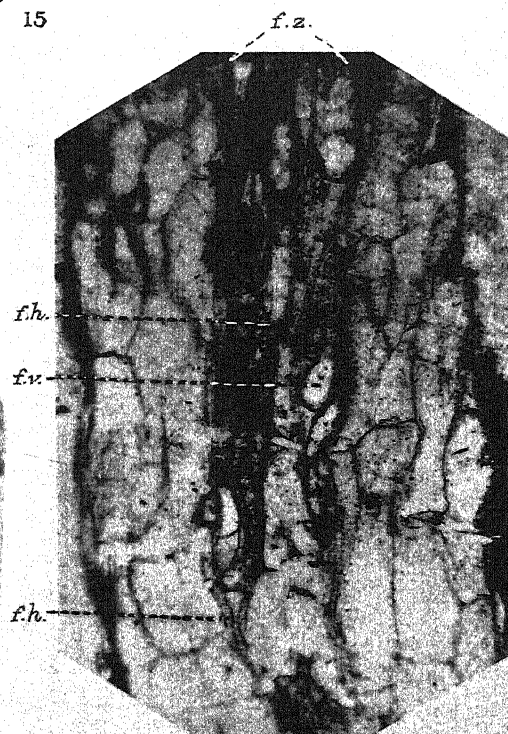
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18

Seasonal Change in the Catalase Content of Conifer Leaves.

BY

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THIS short paper is necessitated by a recent communication by Burge and others (5) which purports to be a reply to an earlier paper on this subject by Doyle and Clinch (4).

In the course of investigations on seasonal changes in the leaves of Conifers the latter authors attempted to further the analysis of the causes underlying the starch \rightleftharpoons sugar equilibrium by a seasonal examination of the leaf enzymes. Rather for completeness, than because any direct connexion was deemed probable, the enzyme catalase came in for consideration. Just at this time a paper by Burge (1) (substantially repeated in other communications which need not be here cited) was noticed in which it was claimed that the leaves of Conifers showed a marked seasonal rhythm in catalase content, the content being markedly high in summer and distinctly low in winter. Several species were claimed to have been studied, but records were published with reference to one only—*Pinus strobus*. These results of course agreed with the thesis so long laboured by Burge and his students that catalase is, at least, an index of metabolic activity. The records published for *P. strobus* looked so convincing that they were accepted at the time, but, merely with the idea of having parallel results available, Doyle and Clinch (4) made some observations on Conifers growing in the Botanic Gardens, Glasnevin, Dublin. Such preliminary results were found to depart markedly from Burge's claims. A systematic series of observations were then undertaken over a period of a year on a number of forms, the results showing so complete a disagreement with those of Burge that no reconciliation between them seemed possible, a maximum, sometimes a very high maximum, being shown in

the winter, a minimum catalase content being characteristic of July. It is as well to note again that Doyle and Clinch, having no particular bias in favour of any theory of catalase function, actually began the observations merely to parallel, under local conditions, those already recorded by Burge. The extraordinary disagreement with the latter's results was thus forced upon the former authors solely by the cogency of apparent facts.

The recent communication from Burge and others (5) is in the nature of a reply. Branches of *P. strobus* in water were placed, in winter, some in the open at an average temperature of -5°C ., some in a greenhouse kept first at 22°C . for 4 days and then at 38°C . for 3 days. The catalase content of those in the open remained substantially constant, while at 22°C . the content was approximately doubled, rising rapidly on transference to the higher temperature to an amount more than eight times the original. These observations are taken as confirming the original findings that cold winter weather decreases and hot summer weather increases the catalase of pine needles, as refuting the findings of Doyle and Clinch to the contrary, and as furthering the claim that whatever changes metabolism in any direction produces a corresponding change in catalase.

Before recording the few additional observations which have since been made, two obvious points need noting. Leaving to one side the debatable question of catalase function, the point at issue is clearly—what actual seasonal change, if any, does catalase undergo in evergreen leaves? Results obtained by artificially transferring leaves, in their winter condition, to an abnormal environment may be, and probably are, of interest in a general consideration of catalase activity but cannot be used to prophesy what the normal sequence will be. It is well known, for instance, that in the stems of many trees starch diminishes or entirely disappears in winter, but that this is not essentially determined by temperature is shown by the fact that cooling of these stems in summer does not induce the starch disappearance, which can only be induced artificially when a certain stage of metabolism has been reached in the autumn. Here is a clear case where the transference at one season to an abnormal environment is no indication whatever of the normal sequence. [Some aspects of this have been dealt with elsewhere (2).] Even granting the correctness of Burge's recent results, they are no contribution to the question of the natural catalase rhythm, if such exists, and, in this sense, refute nothing. A more important point, however, is this. Referring to the work of Doyle and Clinch, Burge says: 'Doyle . . . repeated these (i. e. Burge's) observations, using the same species of pine (*P. strobus*), and obtained results exactly the reverse of ours.' Although not quite wrong, this statement is far from being fully correct. Doyle and Clinch (4) published the full records of five species, of which *P. strobus* was only one. A sample of the results obtained is worth including here.

TABLE I.

Extract of Results on Seasonal Catalase Change already Recorded.

	July.	January.	May.
<i>Tsuga Albertiana</i>	0.8	6.0	2.0
<i>Tsuga canadensis</i>	0.4	30.0	8.5
<i>Juniperus communis</i>	11.5	36.0	20.0
<i>Pinus laricio</i>	0.5	18.5	1.5
<i>Pinus strobus</i>	0.8	2.4	3.8

The numbers represent the total O_2 liberated from 2 c.c. of 20 vol. H_2O_2 by 2 c.c. of leaf suspension.

Estimations for the intermediate months as well as some necessary points of explanation fully appear in the paper from which these are extracted, but always a steady grade from July to January and from January to May and June appeared. Now it is clear that the first four species show a marked winter maximum and summer minimum, but of these Burge makes no mention, only referring to *P. strobus*. Actually the figures for *P. strobus* leaves show a steady increase with age, irrespective of seasonal change, in the first year. It may be mentioned here, however, that in the second year the leaves of *P. strobus* showed a rise from July to January followed by a spring fall, coming then into line with the other forms. In spite of this, Doyle and Clinch specifically called attention to the fact that as far as their records showed *P. strobus* seemed a partial exception to the phenomenon so clearly shown by the others, and suggested that further examination of it might well show that it either behaves without uniformity or even is a complete exception, Burge's claim for it being possibly quite correct at any rate for American conditions.

It seems clear, therefore, that as long as the other species are ignored it is not possible to draw broad conclusions on seasonal changes from the results gained by subjecting *P. strobus* to an abnormal environment. And even granting, as Doyle and Clinch practically did, pending fuller examination, that *P. strobus* behaves as recorded by Burge, as long as it stands alone in its behaviour among many others behaving in an exactly opposite manner, it is not correct to pick it out, to draw conclusions from it, and to exclude the others. That, it would appear, is what Burge has done.

EXPERIMENTAL RESULTS.

During the last half year, in the intervals of other work, opportunity was taken to re-examine a number of the forms already described and to extend the scope to include some new types. Since most of the earlier records dealt with leaves in their first year, the new records rather sought to cover the changes on the older leaves. Granting even the impossible,

that the array of figures already published were due to some incredible coincidence, such coincidence could not be repeated.

A. *Methods.*

The methods used were simple and direct, and have largely been described in the earlier paper. Fresh leaves, 2 grm. in amount, were ground with 1 grm. of precipitated CaCO_3 and a little water, the whole when reduced to the finest state of division being made up to 30 c.c. It is necessary always to adopt the same procedure. If leaves are ground dry with the CaCO_3 or ground wet with a later addition of CaCO_3 and so on the results will vary. The maximum activity, using this method, is got by grinding with the chalk and a little water. It is neither necessary nor advisable to use sand to facilitate the grinding as it renders it difficult to secure small uniform samples later. Two c.c. of the leaf suspension, well shaken of course, are introduced into one limb of a Y-tube, 2 c.c. of neutral 20 vol. H_2O_2 into the other. We have found tubes very convenient of about 12 mm. bore, the limbs about 12 cm. long splayed at 80° , with a 5 cm. long stalk. This reaction tube is connected by means of a T-piece and rubber tubing to a burette, connected in turn with a second burette to be used for maintaining an equal pressure. The Y-tube was immersed in a constant bath at 25°C . When the contents of the reaction tube attained the temperature of the bath, the level in the first burette was suitably adjusted, the T-piece closed, the fluids in the reaction tube mixed and moved from one arm to another at the rate of about once per second. Heinicke (6), who used essentially the same apparatus, states that the total oxygen liberation is the same within fairly wide degrees of agitation. We have found the same result—though the rate will of course vary, the total liberated varies very little. In actual practice, however, the degree of variation in the agitation was very little and the error due to it may be safely disregarded.

In each case the total O_2 liberated—i. e. till the action ceased—was taken as a measure of catalase activity. Of course it is strictly understood that the figures so obtained do not really represent quantitatively—in the sense of accurate analysis—the comparative quantities of catalase present in the different samples. Accurate quantitative comparison cannot be got so simply. But they do approximate relatively in the sense that a larger liberation of O_2 by sample A over sample B represents a larger catalase content in A, although the accurate relative contents are not proportional to the O_2 liberated in the two cases. It is also clearly to be understood that these numbers do not represent maximum O_2 liberation for the quantity of catalase taken. The temperature used is much too high—the O_2 liberation being perhaps twice as great at a temperature nearer to 0°C . At such a temperature, however, the reaction rate is very slow, and when

only a general and not an accurate comparison is required, too tedious. The importance of the temperature effect on the catalase reaction is thus fully realized, but when all samples are tested at the same temperature, although the accurate quantitative relations are further disturbed the higher the temperature, yet the general approximate comparison still holds.

A final point needs notice. After some of the records tabulated below is the symbol $\frac{1}{8}$ or $\frac{1}{4}$ or $\frac{1}{2}$. Now in many cases the catalase content is so high that 2 c.c. of the leaf suspension holds a great deal more than is sufficient completely to break down 2 c.c. of 20 vol. H_2O_2 . In such cases the suspension was diluted to $\frac{1}{2}$ or $\frac{1}{4}$ or $\frac{1}{8}$ strength as necessary. The figure obtained experimentally was then multiplied by two or four or eight. Again, it is to be understood that such procedure is only approximate and not quantitatively accurate, but the same considerations apply here as to the temperature effect. Thus multiplied, however, the figures give, not only the approximate relative seasonal change in each species but also a general idea of the relative catalase activity of the different species.

B. Seasonal Changes in Conifer Leaves.

These are most suitably recorded in a tabular form (Table II, p. 912). Only the change from summer to winter is here dealt with.

Examination of these figures shows that Burge's claim of a fall in catalase activity in evergreen leaves in winter is definitely incorrect. A winter rise has now been shown in nine species; in four of them in two different years. The rise in some cases is spectacular as in *Pinus laricio*, in which species it is not possible to miss noticing the extraordinary increase in winter over summer. It is obvious a fraction of a second after mixing the fluids in the reaction tube. Special interest attaches to *P. strobus* naturally. Very numerous observations were made on it, but in no case was it possible to interpret them in any other light than as indicating an increase in winter. *Abies Webbiana* is of interest also, showing as it does a gradual increase with age irrespective of season and, in this respect, agreeing with *P. strobus* as earlier recorded and as here repeated. The full course of the catalase activity over a longer period must be made clearer, however, in *P. strobus* and *A. Webbiana*.

Observations available show in every case, except these two, a decrease in both young and old leaves with spring. They are held over for the present, however, till more complete and till suitably related with other phenomena. Thus, the October and January records of *Picea omorica* are approximately the same. The October records were taken during the last few days in the month. When followed month by month this phenomenon appears much clearer, a rapid rise, sometimes later, sometimes earlier, being distinct in many cases. The spring fall may show a like sharp

break. The rapid catalase change seems to be associated in some way with the onset of the winter condition or its waning in spring. It seems to be fairly synchronous also with the disappearance in autumn of starch from the leaves and its regeneration in spring, a change which is often quite abrupt. It is hoped to publish data subsequently bearing on such relationships.

TABLE II.

Approx. Catalase Activity of Various Conifer Leaves at Different Seasons.

	July.	October.	January.
<i>Tsuga Albertiana</i> . 1.			
A	0.3	1.5	12.5
B	3.0	5.0	22.0
<i>T. Albertiana</i> . 2.			
A	—	2.5	7.0
B	2.0	6.5	12.0
<i>Juniperus rigida</i>			
A ($\frac{1}{2}$)	24.5	31.0	51.0
B ($\frac{1}{2}$)	26.0	32.0	45.0
<i>J. Communis</i>			
A	54.0 ($\frac{1}{2}$)	64.0 ($\frac{1}{2}$)	170.8 ($\frac{1}{8}$)
B	61.0 ($\frac{1}{2}$)	—	140.0 ($\frac{1}{8}$)
<i>Abies Webbiana</i>			
A	—	7.0	12.0
B	14.5	15.5	20.0
<i>Picea omorica</i>			
A	—	26.0 ($\frac{1}{2}$)	32.0 ($\frac{1}{2}$)
B	10.5	64.0 ($\frac{1}{2}$)	66.0 ($\frac{1}{2}$)
<i>Pinus contorta</i>			
B	1.5	—	10.0
<i>Pinus laricio</i>			
B	3.0	37.0	63.0
<i>Pinus strobus</i>			
A	—	5.0	7.5
B	7.0	11.0	14.0

A = Young leaves and in their first year.

B = Leaves in their second year.

Records given to nearest 0.5 c.c.

If, however, it is clear that there is no winter fall in catalase activity but a marked rise, the question of catalase function in relation to respiration or otherwise is very open. What general physiological function can it have which necessitates a hundred times greater accumulation in the Juniper in January than in the Hemlock in October? Its presence in forms like the Hemlock may be obscured by an inhibitor, just as Doyle and Clinch (3) have shown that peroxidase (in the strict sense of an enzyme blueing guaicum in the presence of H_2O_2) is apparently universally present in Conifer leaves but commonly masked by an inhibitor readily removable by alcohol. No indication of such a catalase inhibitor has, however, yet been found by us. There is a temptation to look upon catalase as a labile

metabolic bye-product rather than as an enzyme with a fundamental physiological function. Future analysis alone will show.

No records seem available of comparative respiration rates during the summer and winter conditions under controlled temperature. Recently Zacharowa (8) studied the gas exchange in *Pinus sylvestris* and *Picea excelsa* during the winter in the neighbourhood of Moscow. The experiments were, however, carried out at low, practically air, temperatures, the CO₂ liberation being naturally very small. His results might be taken to show a slight decrease in respiration in January, irrespective of the temperature. But the real problem has still to be taken up.

C. Effect of Abnormal Temperature Increase on Catalase Activity.

As already indicated, Burge claims a marked increase of catalase over the control in the cold when material is kept at approximately summer temperatures in winter. Since we are still ignorant of the actual function of catalase, such a change might well take place under such conditions. Heinicke (6), for example, reports an increase in cut branches merely on standing. Even though the substantiation of the effect of temperature has no direct bearing on the real seasonal change, it seemed advisable to test the point anew.

In all, four species were tested. Cut branches in water were kept in the open in the shade of a northerly facing wall, similar branches being placed in one of the warm houses in the Botanic Gardens at Glasnevin. The house is not of course under accurate temperature control, but even at night it never fell below 65° F., usually remaining about 70° F. During the day the temperature rose, and with sun, which shone during the mid-day period on every day, except a very few, during which the experiment lasted, a maximum of 93° F. was recorded for a couple of hours. This, while a fairly natural condition of diurnal rise and fall, is still warmer than the average summer conditions at the station. In the open, conditions were very favourable owing to a steady low temperature. The average night minimum was 25° F. (with only two nights above freezing-point), the average day maximum being 43° F. These temperatures while not severe are quite cold for the station, and should afford apparently a sufficient contrast with the warm house to ensure a repetition of the Burge effect, even though the difference in his range was much wider. The catalase activity of the leaves was tested in each case when the branches were set and again after a period of five to eight days at the two temperatures. The results appear in the succeeding table. The estimations were made in the same manner as before. The numbers bear the same meaning as before.

These results caused us considerable surprise, as we fully expected to meet the temperature effect described by Burge for *P. strobus*. The extraordinary constancy of the results, considering the extremes of conditions

to which the branches were subjected as well as the nature of the method, is really remarkable. It is perhaps a pity that *P. strobus* is not included in the results. The only specimen available in the Botanic Garden is not in good condition, and it was feared that the removal of a sufficient number of large branches for a satisfactory test might injure the tree. Also it is very easy in most pines to block the vessels with resin from the cortical ducts in cutting the shoots. It is hoped to deal later with growing potted plants. But even if the rise of catalase with increase of temperature were substantiated for *P. strobus*, the results from the four trees here examined show that it can only be an exceptional case, and that general conclusions cannot be drawn from it.

TABLE III.

Effect of Abnormal Increase of Temperature on Catalase Activity.

A = sample in open. B = sample in warm house.

In each case the first date = when set up, the second date = when removed.

	A.	B.
<i>Picea omorica</i>		
24/1/30	22.0	25.0
29/1/30	23.7	28.5
<i>Tsuga Albertiana</i>		
30/1/30	8.6	8.5
6/2/30	9.3	10.1
<i>Tsuga Albertiana</i> (a different tree)		
6/2/30	11.0	11.3
14/2/30	10.5	11.1
<i>Taxus baccata</i> ¹		
15/2/30	61.0 ($\frac{1}{2}$)	61.0 ($\frac{1}{2}$)
20/2/30	59.0 ($\frac{1}{2}$)	60.0 ($\frac{1}{2}$)

CONCLUSION.

The further evidence presented shows in all the species examined, that the catalase content rises in winter and remains unchanged during that season even when the plants are subjected to a higher temperature for a short while. Induced changes in the content may possibly be obtained in the future by suitable experiment, but there is no evidence available in support of Burge's theory of the close relationship between catalase and general metabolism as far as Conifers are concerned. Whatever value may be placed on his own results with *P. strobus*, comparison with other forms is obviously necessary before general conclusions can be drawn, and that

¹ The numbers for *Taxus baccata* are high. The reaction with this species was allowed to proceed at 13°C., 2 c.c. of 40 vol. H₂O₂ being used.

comparison Burge did not make. It is perhaps of interest to note the opinion held of the theory by animal physiologists. Morgulis (7), in his recent summary of our present knowledge of catalase, speaking of Burge's work, says: 'Mehr als 40 in der Literatur erwähnte Arbeiten, die alle aus demselben Laboratorium stammen, sind so durch und durch spekulativer Art, in ihrer Einfachheit so naiv, dass es schwierig ist, Phantasie und Tatsachen auseinander zu halten. . . . Die Übereinstimmung und die nie versagende Genauigkeit der Resultate der Versuche, in welchen er seine Theorie prüfte sind nicht weniger überraschend. Dank seinen Voraussetzungen lässt sich sein Versuchsergebnis ohne Fehler voraussagen.' As it is hoped to deal more adequately later with the question of catalase in relation to seasonal change, this note is only of a provisional nature, with the aim of insuring that, as far as this question is concerned at all events, fact will rule, no matter what the fact may be.

We make the usual sincere acknowledgement to J. W. Besant, Esq., Keeper of the Botanic Gardens, Glasnevin, Dublin, for continued permission to make use of the facilities in the Gardens.

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The Biology of Banana Wilt. (Panama Disease).

II. Preliminary Observations on Sucker Infection.

BY

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With seventy-four Figures in the Text.

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I. INTRODUCTION.

IN Part I of this work, the author has expressed the opinion that in the production of the major symptoms of Banana wilt, the problem of fundamental importance is that of sucker infection. Root disease, while important in the general physiology of the plant, has been shown to be of a variable nature, and is regarded as being of chief importance when it occurs in proximity to the sucker (9).

In the inoculation experiments of Brandes (1), and Reinking (7), while definite proof of the pathogenicity of *F. cubense* to the Gros Michel has been

obtained, their accounts contain little or no information regarding the actual behaviour of the attacking organism when brought into contact with its host. Again, in the several papers there is little detailed information regarding the nature and rate of infection, the state of the sucker from time to time, or the influence of external factors. In short, one is left with the impression that the sucker has only to be exposed to the organism to become diseased, and that the infection is rapid, straightforward and unimpeded. But general experience indicates that, almost without exception, the several important wilt diseases are both complicated and variable. These remarks are true also of Panama Disease.

In a series of preliminary experiments, to be recorded in this paper, the author set out to investigate the behaviour of the sucker during the early stages of infection, and, if possible, to determine the nature and rate of fungal penetration. These experiments have proved exceedingly useful and have brought to light a mass of data hitherto unrecorded regarding the reactive power of the sucker in relation to the penetration of parasitic organisms.

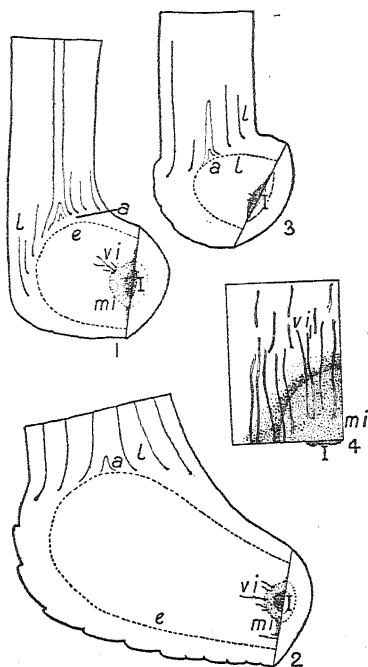
Note.—The banana has a condensed but profusely branched underground stem or rhizome. Planting material, for plantation purposes, consists of (a) 'bits', i.e. portions of the rhizome provided with buds; (b) 'suckers', which are simply rhizome buds more fully developed; suckers may be detached by cutting through with a spade or cutlass without uprooting the whole rhizome. Such a detached sucker consists of a basal storage stem, or rhizome, and an apical portion, in which the typical sunken monocotyledonous meristem is enveloped in leaves. Suckers of this kind, with only one cut surface, were used throughout in these experiments.

II. INOCULATION OF SUCKERS IN MOIST CHAMBERS.

A first series of observations on sucker infection was made as follows. Suckers of various sizes from a disease-free area were sterilized superficially with 0.2 per cent. mercuric chloride solution, and washed twice in sterilized water. Tin boxes with press-on lids, of 4,000 c.c. capacity, were also sterilized as above, and provided with a large petri dish surrounded by sterilized water. This made a fairly suitable moist chamber for preliminary experiments. The sterilized suckers, suitably trimmed, were then cut across transversely at the basal end with a sharp flame-sterilized knife. The cut end of each sucker was then carefully examined for any blemish indicating decay or the presence of parasitic organisms. The disease-free suckers were then inoculated in the centre of the cut surface with a small inoculum of *F. cubense* growing on a solid agar medium. One, two, or more suckers were placed in each box according to size. The lids were

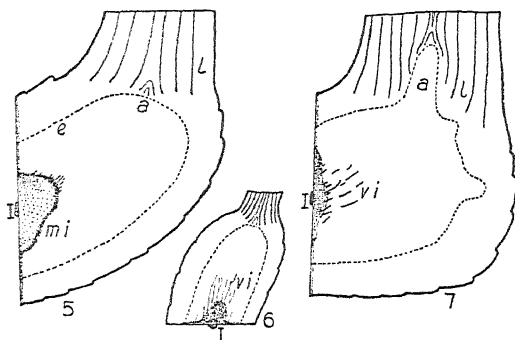
then adjusted. In the field, the disease may sometimes take two, four, six, or more months to make its appearance; that is to say, sucker infection is comparatively slow. In order to speed up the rate of penetration, therefore, it was found convenient to use, in these first observations, unperforated lids. The infection was thus allowed to proceed in an atmosphere vitiated by the carbon dioxide liberated during respiration. A noticeable amount of penetration, 1.5 cm., took place in ten days. Characteristic records for Gros Michel suckers are shown in Figs. 1, 2, and 4, and for a Canary banana sucker, *M. cavendishii* (resistant variety), in Fig. 3. At the time of examination, the suckers were cut through longitudinally, and the depth of penetration observed. At this stage the infection was found to be what may, for convenience, be described as a *mass infection*, in contrast to its later stages, when it is found almost entirely as a *vascular infection*. Even at this early stage, however, it was observed in some suckers that, passing out from the region of *mass infection*, there were vascular strands showing the characteristic yellow and red discoloration typical of Panama disease, extending about 1.5 cm. inwards. In the mass infection, which was of a dark brown to black colour, several zones could be observed megascopically (Fig. 4). Detailed observations will be set out later, and it will suffice here to mention that next the inoculum there was a well-marked soft and spongy zone, obviously being exploited by the hyphae, while further in was a zone of discoloured tissue of firmer texture, where killing in advance was taking place by the diffusion of toxic secretions from the fungal metabolism.

Dead banana tissue is easily recognized, since the oxidases liberated by killing act on the cell contents, producing a purplish-blue colour. Some suckers of the highly resistant Canary banana (*Musa Cavendishii*) were also inoculated and under the conditions of the experiment (as before) a slight penetration was obtained in ten days (Fig. 3). The experiment

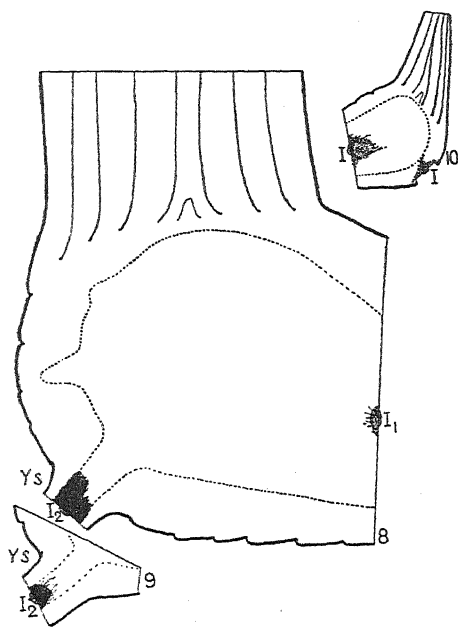


FIGS. 1-4. Figs. 1 and 2. Gros Michel suckers; Fig. 3. Canary banana sucker; inoculated at 1, with *F. cubense* and kept in moist chambers for 10 days. Penetration takes place *en masse* at first (*mi*.) then gradually develops into a vascular infection (*vi*.). The suckers are shown in longitudinal section ($\times \frac{1}{2}$) *e* = endodermis; *a*. = apex; *l*. = leaf bases. Fig. 4. Gros Michel: region of mass infection (*mi*.) with infected vascular strands passing out (*vi*.). $\times 3$.

was continued with other suckers for twenty-five days. Records for small and larger Gros Michel suckers are shown in Figs. 5, 6, and 7. In Fig. 5,



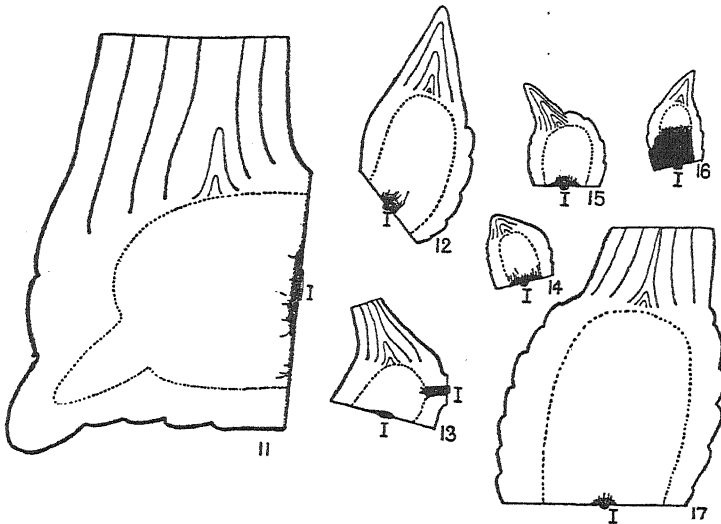
FIGS. 5, 6, and 7. Gros Michel suckers (in longitudinal section) inoculated with *F. cubense* and placed in moist chambers for 25 days. I. = inoculum; *m.i.* = mass infection; *v.i.* = vascular infection; *e.* = endodermis; *a.* = apex; *L.* = leaf bases. $\times \frac{1}{2}$.



FIGS. 8, 9, and 10. Gros Michel suckers (in longitudinal section) inoculated with *F. cubense* and kept in moist chambers for 12 days. I. = inoculum; *I*₁ = inoculum on adult tissue, shallow penetration; *I*₂ = inoculum on young sucker (*y.s.*) deeper penetration. Fig. 10 shows the deeper penetration at the basal end of a young sucker, and more shallow penetration at a lateral wound. $\times \frac{1}{2}$.

mass penetration had continued so that the sucker in longitudinal section showed a deep blemish round the inoculum. In the smaller sucker shown in Fig. 6, mass infection had not progressed so markedly, but a considerable amount of vascular infection had taken place. In Fig. 7, mass pene-

tration was not very deep, but a considerable amount of vascular infection and discoloration had again taken place. These records serve to indicate the variable results obtained. While further observations under these conditions would have been interesting, this was not practicable on account of the development of leaves at the apex of the suckers.

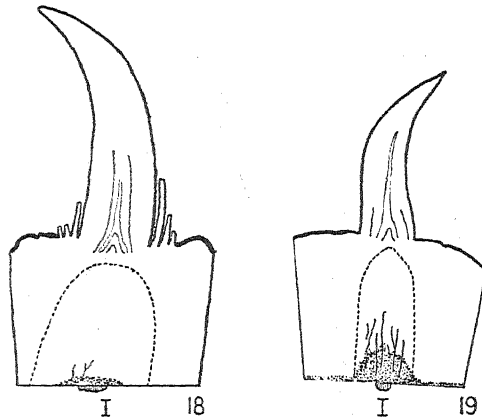


FIGS. 11-17. Canary banana suckers inoculated with *F. cubense* and kept in moist chambers. Fig. 11. Old sucker, after 17 days with shallow mass and slight vascular infection. Figs. 12-16. Deeper penetration into young suckers after 15 days. I. = inoculum. $\times \frac{1}{3}$.

For exact experimentation it is almost impossible to obtain a supply of uniform suckers. Thus, while suckers may be of even size, they may represent very different stages of growth, storage, or maturity. In the experiment described above, it was found, as indicated by Figs. 5, 6, and 7, that different amounts and kinds of penetration take place, i. e. deep or shallow, mass or vascular. To determine the relative nature of infection in suckers of different ages, three lots of six suckers, with well-marked differences in size, were inoculated as before under uniform conditions and examined after fifteen days. It was found that the younger the sucker the greater was the amount of penetration and blemishing.

This was further demonstrated as follows. Large suckers, four to five inches in diameter, from which young suckers were emerging, were selected. The young suckers were cut through transversely at the point of emergence. These surfaces (of meristematic tissue), and also the cut basal end of the large parent sucker, were inoculated with *F. cubense*. On examination, after twelve days, it was found that penetration into the young tissue of the daughter suckers was much greater than into the older basal tissue of the parent sucker. Typical records are shown in Figs. 8, 9,

and 10, where a comparison of the amounts of penetration can be made. The well-known resistance or immunity of the Canary banana (*Musa cavendishii*) to wilt disease, has already been mentioned. As indicated by Fig. 3, however, under these experimental conditions, a certain amount of



FIGS. 18 and 19. Canary banana. Young suckers cut out from parent sucker *en bloc*, and inoculated with *F. cubense* on the inner surface. I. = inoculum. Fig. 18. After 12 days. Fig. 19. Considerable penetration, mass and vascular, after 21 days. $\times \frac{1}{2}$.

penetration does take place. This, however, on many trials, was always found to be much slower and more shallow than in the case of suckers of Gros Michel. Some results of inoculating *F. cubense* on Canary suckers are shown in Figs. 11–17. Again, it will be seen that penetration into young suckers (Figs. 14, 15, 16), is much more rapid than into the older ones (Figs. 12, 17), while penetration into the adult tissue at the basal end of old suckers is very slight, even after seventeen days (Fig. 11). That a considerable amount of penetration may be obtained, even with the Canary banana, was further indicated by the following experiment. Very small emerging suckers were cut out from the parent sucker in the form of a cube of tissue. On the innermost face of the cube the stele of the daughter sucker was thus exposed in transverse section. Several such pieces were inoculated on the innermost face (i.e. on the stele). After twelve days a small amount of penetration, partly mass, partly vascular, had taken place (Fig. 18), and on further examination of other suckers after twenty-one days, a considerable amount of mass penetration was observed, while vascular discoloration was also well marked (Fig. 19). In both Gros Michel and Canary suckers it was found that vascular discoloration was most pronounced at the periphery of the stele.

III. PRELIMINARY ANATOMICAL OBSERVATIONS.

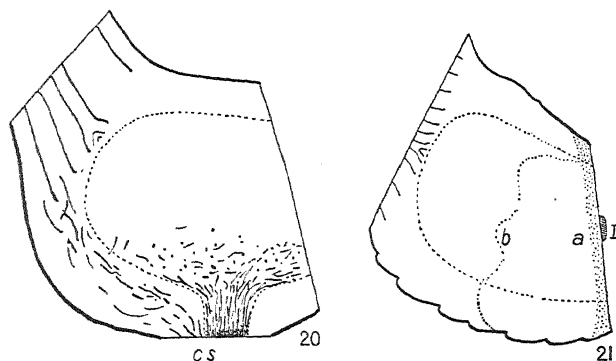
Gros Michel material from the foregoing inoculation experiments was next examined microscopically. For this purpose, longitudinal sections through unfixed tissue were prepared and mounted in water. It was found that hyphae were present only in the outermost part of the affected tissue, the remaining innermost portion being free from hyphae, having evidently been killed in advance by the diffusion of fungal secretions. The behaviour of *F. cubense* in producing these mass infections thus appears to be in agreement with other relatively non-specialized parasites (2). In *Botrytis*, for example, it has been shown that once penetration of the superficial tissue of the host plant has taken place, the parasitism proceeds by a process of killing in advance for which the rapid diffusion of a toxic enzyme is responsible. The dead tissue is then invaded by hyphae which absorb their food materials saprophytically. In banana tissue death is accompanied by the liberation of a bluish-purple stain, a feature of great assistance in determining the extent of lethal fungal activity. Beyond the discoloured tissue obviously affected by the presence of *F. cubense*, another characteristic zone was observed. Here obvious modifications were taking place including (a) removal of storage starch, (b) suberization of walls, and (c) formation of a cambiform layer.

It would appear that the extent of a mass infection is directly related to the rate of diffusion of lethal substances liberated by the penetrating fungus. This will be subject to a number of modifying internal and external factors. Internal factors will refer to the physiological state of parenchymatous tissues in relation to the control of diffusing secretions, i. e. to their ability to react quickly to wound stimuli by the formation of suberized cambiform barriers. The reactive power of root-cells, in the formation of such barriers has already been seen (9). The rapidity with which they are formed is obviously important. This will depend on the age of the tissue, its unspecialized nature and general physiological state. The rate of diffusion will depend on the nature of the substances involved. What these are in the case of *F. cubense* growing on banana tissue has not been determined. Rosen (8) found that *F. vasinfectum* (the causal organism of Cotton wilt) grown on Richard's solution, produced at least two substances toxic to cotton plants. One was a volatile compound with an alkaline reaction, the other an inorganic salt in the form of a nitrite. A comparable concentration of chemically pure sodium nitrite was found to be markedly toxic to cotton plants.

That the rate of diffusion of toxic substances is slowed down by the controlling activity of the living tissue, was indicated by the following two experiments:

- (1) Some Gros Michel suckers were cut transversely under water and

placed in methylene blue or eosin solutions. After twenty-four hours, a marked penetration in the vessels (Fig. 20), had taken place. On the other hand, similarly treated suckers, when placed in staled solutions, in which *F. cubense* had been growing for fourteen to twenty days, only showed a slight penetration of vessels and ground tissue at the end of four days.

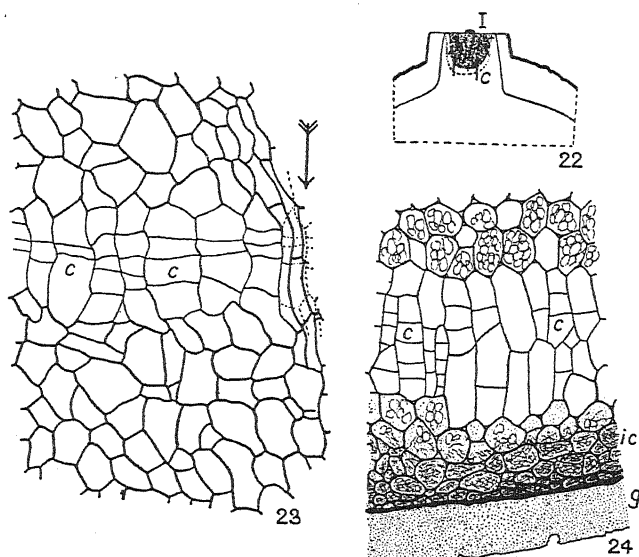


FIGS. 20 and 21. Fig. 20. Gros Michel sucker which was placed in a solution of eosin at the point where a young sucker was cut off. c.s. = cut surface immersed in eosin. The black lines indicate the vascular strands which were stained after 24 hours. $\times 1\frac{1}{2}$. Fig. 21. Sucker of Gros Michel, cooked in the autoclave and inoculated with *F. cubense* at 1 (a) Zone of hyphal invasion, (b) zone of discoloration due to diffusion of fungal secretions after 10 days. $\times 1\frac{1}{2}$.

(2) Gros Michel suckers were placed in the autoclave at 120°C . for forty minutes, and inoculated at the basal cut end in the usual way. After ten days it was found that the sucker, in longitudinal section, had the appearance shown in Fig. 21. About half a centimetre from the inoculated surface there was a zone of tissue where the influence of the fungus was strongly marked (a). There was also a much more extensive zone of slightly discoloured tissue about 4–5 cm. deep (b). The discoloration was found to be associated with the peripheral cytoplasm and cell-walls. In the first region, (a), on treatment with iodine it was found that three zones could be observed. (i) Zone with hyphae, where the cells gave a blue coloration (starch solution), but where the starch grains had disappeared; (ii) Zone where secreted diastase was just bringing about the solution of the starch grain; (iii) Normal cells with swollen starch grains (due to autoclaving). The line in the illustration (a) marks the point where the grains were just beginning to disappear. Thus at least two diffusing secretions appear to be at work, namely, diastase acting slowly on the starch grains, and a much more rapidly diffusing substance capable of causing tissue discoloration. This rapid diffusion was never observed in the numerous sucker inoculations already described.

During the experiments described in Section II it was observed that while penetration was rapid in the first ten days, the amount of decay after

twenty-five days was not proportionately great. Detailed microscopic examinations then showed that the suckers had commenced to react to the parasite by the development of cambiform barriers. Fig. 22 shows a sucker, inoculated at the point of separation of a small daughter sucker, as seen in longitudinal section after being kept for twenty-one days in a

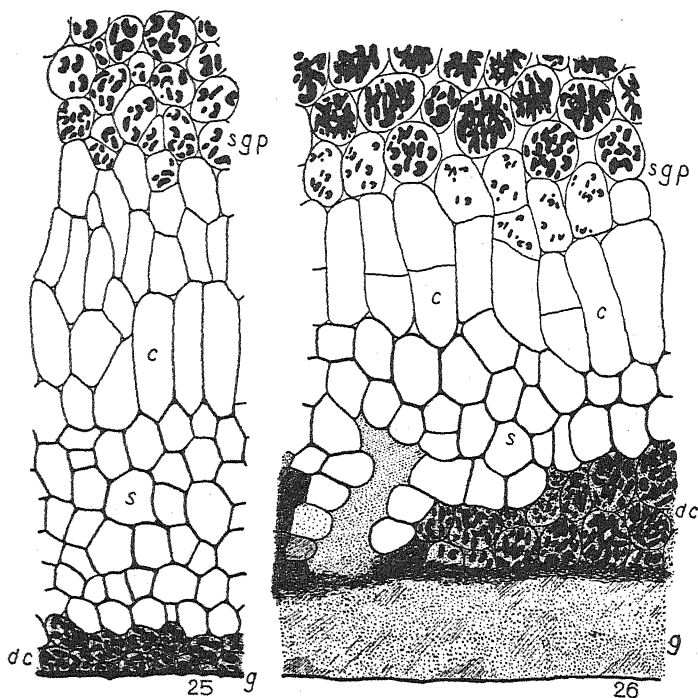


FIGS. 22-24. Fig. 22. Gros Michel; inoculated young sucker after 21 days in moist chamber. $\times \frac{1}{2}$. Fig. 23. A portion of Fig. 22 magnified; cambiform layer (*c*) is being formed from the ground parenchyma. The arrow indicates the direction of diffusion of toxic fungal secretions. $\times 90$. Fig. 24. Longitudinal section from an older inoculated Gros Michel sucker showing gummy outer layer (*g*), infected cells (*ic*) with profuse hyphae, and protective cambiform layer (*c*) which is holding the invasion in check. $\times 50$.

closed moist chamber. Megascopically a semicircular blemish, about 1.5 cm. deep, extending from the inoculum, had been produced. Indications of vascular penetration had also commenced to appear. An obvious feature, however, was the definite delimitation of the discoloured zone by the presence of a thin layer of more translucent tissue, which longitudinal sections showed to consist of a thin-walled cambiform formation. A detailed study is shown in Fig. 23. Hyphal penetration and diffusion of toxic substances were taking place in the direction of the arrow, i. e. from the apical tissue backwards. Again, it was found that cells killed by lethal secretions extended a considerable distance beyond those which had actually been invaded by the hyphae. Here then, in spite of the accumulation of CO_2 , a definite, though belated, reaction to fungal invasion had taken place. Fig. 24, illustrating a similar protective barrier in an older sucker shows that the fungus has been confined to the outermost cells.

IV. THE INFLUENCE OF FUNGAL SECRETIONS.

Having shown that killing takes place in advance by the diffusion of toxic secretions from hyphae growing in banana tissue, and also that



FIGS. 25 and 26. Fig. 25. Longitudinal section through a Gros Michel sucker placed for 4 days in staled malt extract solution. *g*. = gummy layer; *dc*. = dead cells, killed by contact with toxic fungal secretions; *s*. = cells with suberized walls from which starch has been removed; *c*. = cells enlarging to form the cambiform layer; *s.g.p.* = normal storage ground parenchyma. $\times 90$. Fig. 26. Do., from another sucker; transverse septations of the cambiform cells have commenced to appear. $\times 90$.

certain protective reactions take place in the sucker itself, the next stage was to test the direct effect of staled culture solutions.

For this purpose, four staled solutions were used.

1. Three per cent. malt extract solution, staled by the growth of *F. cubense* for twenty-four days.
2. Banana juice extract, prepared by boiling 200 grammes of chopped sucker in 1,000 c.c. water and filtering; staled for twenty days.
3. Asparagin rich culture medium, staled for twenty days.
4. Glucose rich culture medium, staled for twenty days.

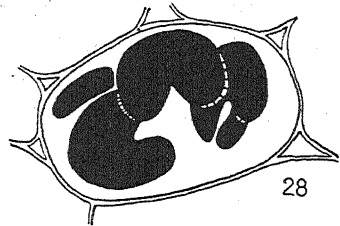
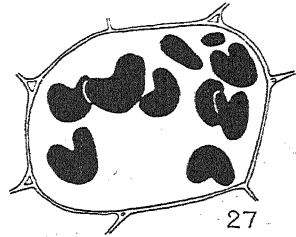
The mycelium and spores were filtered off, and freshly cut suckers of Gros Michel were immediately immersed in the solution and kept there for four days, after which they were examined in longitudinal section.

The resulting tissue discolorations were not extensive, 0.5 cm. being the maximum. As before, it was found that penetration was greatest in the younger suckers. This was readily demonstrated in individual suckers by cutting them obliquely, so that a range in maturity of tissue was exposed to the staled solution. An ascending gradient of penetration was found from the older to the younger tissue.

In order to gain some idea of the activity of the staled solutions relative to a definite fixing solution suckers were placed for twenty hours in 0.2 per cent. mercuric chloride solution. An average penetration of 1.5 cm. was found indicating the much slower and less lethal action of the staled solutions.

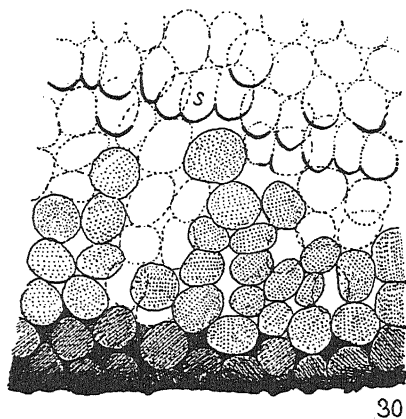
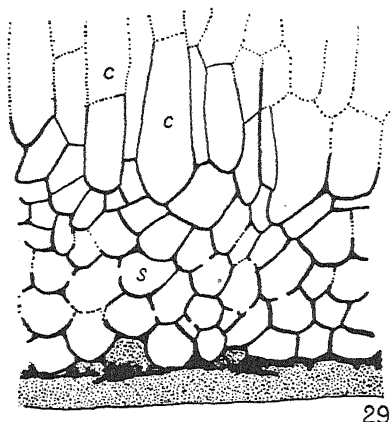
Longitudinal sections through tissue blemished by immersion in staled malt solution were examined in chlor-zinc-iodine (Fig. 25). On the outside, in contact with the staled solution, there was a thin layer of exuded gum. Immediately inside was a double layer of cells containing starch grains which had been killed by contact with the staled solution. Proceeding in-

wards, this was succeeded by a layer of cells, five to seven cells deep from which all the starch had been removed. The cell-walls had become partially suberized. This was followed by a zone of tissue where the cells had elongated, prior to the formation of a cambiform barrier. Here also the starch grains had been removed. On the inner limit of the developing cambiform-layer was the normal storage parenchyma, with transition cells containing very small and scanty starch grains (Fig. 27), in contrast to the large grains of the normal storage tissue (Fig. 28). It was at first thought that the removal of starch from the outer suberized and cambiform tissue was due to the activity of diastase present in the staled solution. That this was not so, however, was definitely indicated by the fact that the cells immediately abutting on the solution were still fully packed with large starch grains; these would normally have been the first to be dissolved. The disappearance of starch, therefore, is a reaction of the living cells to wounding and to the diffusion of fungal secretions. The exact utilization of the starch by the plant under these circumstances is worthy of careful investigation since its disappearance in solution has been demonstrated to be an essential feature and definite concomitant of the formation of the protective barriers. The suberized cells were examined in detail by



FIGS. 27 and 28. Fig. 27. Small starch grains in cells abutting on the inside of the cambiform layer. $\times 370$ Fig. 28. Normal starch grains of unmodified ground parenchyma. $\times 370$.

dissolving away the cellulose tissue in concentrated sulphuric acid. Suberization extended to the outer walls of the cambiform tissue. The intercellular spaces of the suberized zone were frequently found to be filled with a yellow-coloured gummy exudate which also was not dissolved by concen-



FIGS. 29 and 30. Fig. 29. Longitudinal section of Gros Michel sucker placed in staled banana juice for 4 days. The section is taken from tissue near the meristem. $\times 50$. *s.* = cells with suberized walls with starch removed; *c.* = cambiform cells. Fig. 30. Older tissue of same sucker. The outermost cells are incompletely suberized. Further in, at (*s.*), a new deposition of suberin is in progress. $\times 50$. Both illustrations were prepared from sections placed in concentrated sulphuric acid to remove the cellulose. Dotted lines indicate dissolved cellulose walls.

trated sulphuric acid. A second section prepared from the peripheral region of the stele of another sucker placed in staled malt solution is shown in Fig. 26. This shows the general succession already described, with the difference that the cells of the cambiform layer have just undergone their first transverse divisions. Beyond this were normal cells containing a few small starch grains. The appearance of the latter suggests that solution takes place layer by layer. Further sections from this material showed that the reaction of the tissue varies in different parts of the sucker, different degrees of suberization being observed. Immersion of sections in concentrated sulphuric acid showed that the starch-containing cells at the cut surface were not suberized. The cells further in, however, had been able to react sufficiently quickly to effect starch removal and suberization. Sections from one sucker immersed in staled banana extract were found to have two to six layers of suberized cells, but no starch removal or cambiform formation. A second sucker, cut obliquely so as to expose tissues of different maturities, showed a range in reactions. The youngest tissue, i. e. nearest the meristem of the sucker, in longitudinal section, showed the following zones: (*a*) a gummy layer; (*b*) a layer of starch-containing cells killed by the toxic solution; (*c*) four to six layers of suberized cells, containing no starch; (*d*) a single, double, or treble layer of cambiform cells,

some of which had commenced to divide by transverse walls; (*e*) cells of normal size containing a few small starch grains; (*f*) normal starch-filled parenchyma.

When such sections were immersed in concentrated sulphuric acid, the results were as illustrated in Fig. 29, where it will be seen that suberization has proceeded right up to the outer walls of the cambiform cells, partly along the longitudinal walls, and sometimes across, or partly across, the transverse walls. Suberization of the outermost parenchyma was frequently observed to be incomplete.

Longitudinal sections from older parts of the same sucker showed no starch disappearance or cambiform tissue. It has already been mentioned that mass penetration was decidedly less in older tissue. When the longitudinal sections were placed in concentrated sulphuric acid, the outermost cells (Fig. 30) resisted the solvent action of the acid. The innermost perceptible limit of stimulation was indicated by a single or double layer of cells, whose walls were just becoming suberized on the side towards the advancing secretions.

Between the conditions present in the older and younger tissues described above, all intermediate stages in starch removal and cambiform activity were found. It would thus appear that while young sucker material is more deeply penetrated, it is also, as might be expected, more highly reactive. Similar observations were made on suckers which had been immersed in the staled asparagin-rich medium, but on the whole the changes induced appeared to take place more slowly. A young sucker, cut transversely about 1 in. from the apex, and immersed in the staled sugar-rich solution for four days, showed some interesting features. Longitudinal sections showed the following succession from the outside inwards. (*a*) Layer of exuded gum; (*b*) a layer some six cells deep, killed by the toxic solution, and with starch grains not removed; (*c*) a deep layer from which all the starch had disappeared, slightly suberized next the dead cells of (*b*); (*d*) a second zone of slight suberization or none; (*e*) normal starch containing tissue. A second sucker showed no trace of starch removal. The outermost tissue, one or two cells deep, resisted the solvent action of H_2SO_4 . The adjoining inner tissue was unsuberized for a distance of 1.5 mm. (i. e. over 25-35 cells), and this was followed by a layer of parenchyma whose outer walls were just becoming suberized (Fig. 31). These observations suggest that there is a close contest between the rate of diffusion of toxic secretions and the development of protective systems on the part of the plant. There is also evidence that the substances described here as suberin (i. e. those giving the several characteristic colour reactions and resistant to concentrated H_2SO_4) do not always prevent the passage of toxic substances. This may be due to the rapid diffusion of the latter into cells before the formation of

true impervious suberin is fully completed. As Priestly (6) has pointed out, suberin is a collective name for a number of closely allied substances, the final condensation product being the impervious cell-wall substance.

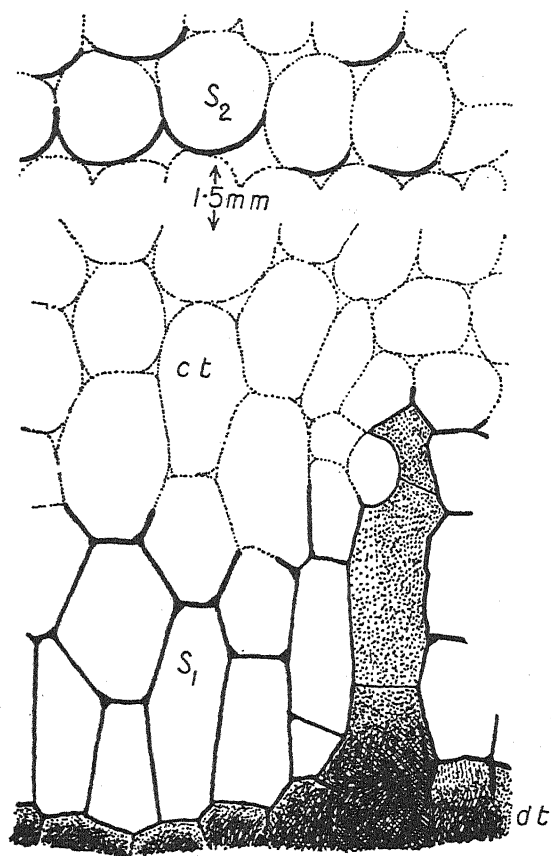


FIG. 31. Longitudinal section of Gros Michel sucker placed for 4 days in staled sugar solution. $\times 186$. *dt.* = dead tissue in contact with toxic solution; *s₁*. = incompletely suberized cells which have not held up diffusion of fungal secretions; *ct.* = cellulose tissue; *s₂* = commencement of suberin deposition in a more deeply seated layer of cells. Drawn from a sulphuric acid preparation in which all the cellulose has been dissolved; the latter is indicated by dotted lines.

The observations set out in this section, though purely of a preliminary nature and capable of much amplification, are significant in that they indicate (*a*) the importance of fungal secretions, and (*b*) the ability of healthy sucker tissue to react to the presence of irritant or toxic substances.¹ A comparative study of the rates of formation of protective barriers under different external conditions, and for different varieties of banana, will be taken up later.

¹ In a paper by F. Seto (Rev. Appl. Myc., viii, p. 197, 1929), a *Fusarium* disease of rice is described in which inoculation did not check growth but stimulated the rice seedlings to abnormal development.

V. PENETRATION OF *F. CUBENSE* INTO GROWING SUCKERS.

Several experiments were set up to test the nature and amount of penetration into Gros Michel suckers when placed in soils of different hydrogen-ion concentration—a range of pH 5.8–pH 8.1 being covered. A silt soil, on which Panama Disease notoriously occurred, with pH 6.4, was used in this experiment for purposes of comparison. As the other soils used were of a rather intractable and sticky consistency, about 30 per cent. of sand was intimately admixed. The soils were not sterilized. Using these soils, suckers were potted up in 10-inch pots, set in pans of water, and watered daily from above. Each pot was heavily inoculated before planting with several strains of *F. cubense*. The basal end of each sucker was cut clean across with a sharp knife before planting. Altogether some two dozen suckers grown under these conditions were examined after growth periods varying from 50–110 days. The roots described in Part I were taken from these pots, and it has been seen that, with a few exceptions which are described in detail, they were healthy and free from infection although exposed to the fungus from the outset. The suckers were also found to be very little affected. Mass invasion of the cut basal end was, in all suckers examined, found to be limited in extent, and less in amount than that produced in closed moist chambers in one third of the time. Vascular infection was also found to be inextensive. Thus, in the first two or three months of exposure to *F. cubense*, under the healthy conditions of pot culture, the amount of penetration was singularly small. The experiment therefore afforded a definite record of the tardy initial penetration under such conditions. In the inoculation experiments of Brandes (1), on the other hand, several of the young plants growing in inoculated soil were severely stunted almost from the start. ‘Two of them died before they were one foot tall, but owing to their extremely slow growth, this was a matter of about two months’. Eight months from the time of planting, all of his plants in inoculated soil showed unmistakable signs of disease. Reinking (7) states that in his experiments the time of appearance of disease after inoculation, varied from two to seven months or longer.

The observations set out in detail in the next Section show that the slow initial penetration observed by the present writer is referable to the formation of protective barriers when suckers are grown under conditions of uniform water supply and sufficient aeration.

VI. DETAILED OBSERVATIONS ON PENETRATION OF SUCKERS.

(a) *Limited sucker penetration.* Suckers growing in pots containing a silty soil, pH 6.4, notorious for Panama Disease were examined after ninety days. The aerial parts of the plant were green and healthy and

free from characteristic disease symptoms, as also were the roots. When suckers were examined in longitudinal section penetration at the basal cut end was found to be very slight, not more than 0.5 cm. on the average, with occasional regions of deeper injury, 1 cm. at most. A certain amount

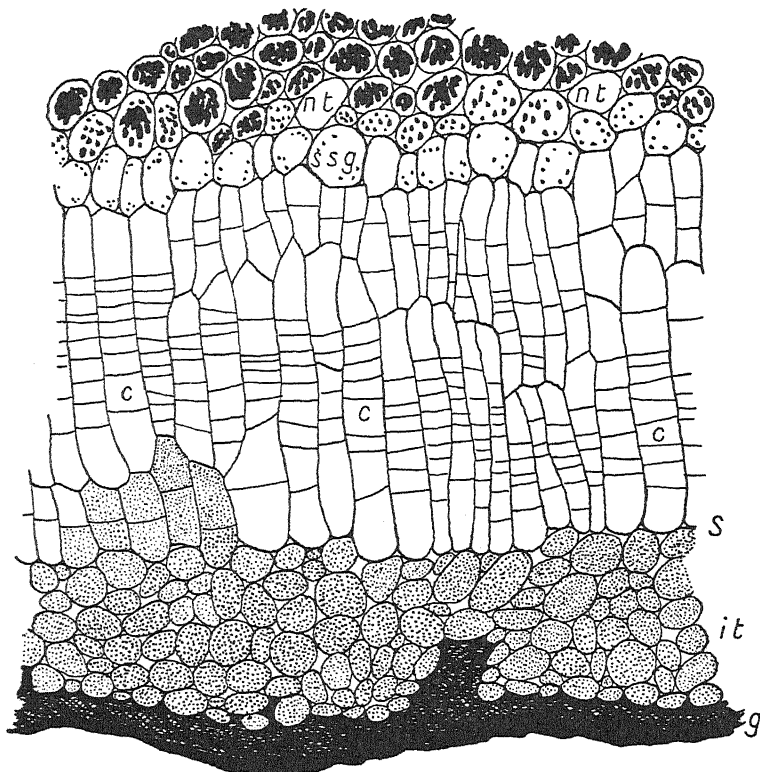


FIG. 32. Longitudinal section through basal cut end of Gros Michel sucker grown in inoculated soil for 90 days. *g.* = gummy outer layer; *it.* = invaded tissue with profuse hyphae; *s.* = suberized outer walls of cambiform tissue (*c*); *s.s.g.* = cells with small starch grains; *nt.* = normal tissue with large starch grains. $\times 70$. The limited penetration, due to the suberized cambiform barrier, is clearly indicated.

of vascular discoloration, extending inwards 1 to 1.5 cm. had also taken place. This constituted the full extent of fungal penetration in any of the suckers after three months. Megascopically it was observed that the spongy diseased tissue was bounded on the inside by a hyaline layer which detailed examination showed to be a well-developed cambiform tissue. The *camera lucida* drawing of a typical section (Fig. 32), shows a decayed gummy outer zone, adjacent to which there is a layer of brown-coloured parenchyma which has been exploited by numerous hyphae. This, however, is of limited extent, and is bounded by a well-developed cambiform layer with eight to twelve septations. The outer walls abutting on the invaded parenchyma are strongly suberized. The cambiform

tissue has been formed by the longitudinal expansion of one or two layers of parenchymatous cells, followed by a series of transverse septations. The resulting formation thus resembles typical cambial tissue. Sections cut from living material and mounted in water, showed that, while the invaded cells were of a deep brown colour, the cambiform layer was clear and translucent, and that hyphae, profusely present in the former, were definitely absent from the latter. A definite proof that this formation is not only effective in holding up hyphal invasion, but also prevents diffusion of toxic fungal secretions, is thus provided. It has already been pointed out that there is a race between the diffusion of lethal substances and the development of impervious protective walls. A typical example is shown on the left-hand side of Fig. 32. Here, four cells have been in the act of assuming the typical elongated cambiform shape, and have divided transversely once, but have been killed presumably before the deposition of suberin was effected. The further advance, however, has been checked by the next innermost layer. The same illustration also shows a cambiform cell, only the outer part of which has been killed, owing to the suberization of the transverse wall. Hyphae were sometimes observed in some of the dead cambiform cells. There is the possibility, of course, that hyphae may be able to force their way through the suberized walls, or that small unsuberized pores may sometimes be left which would afford a passage. The writer, however, has never observed the progressive cell-to-cell invasion of such cambiform barriers. Modern opinion also appears to favour the view that suberized walls are generally without perforations (6). The suberization of successive transverse walls in the cambiform layers would render any attempt at penetration exceedingly slow.

Inside the cambiform layer parenchymatous cells with small starch grains are the rule. Further in lies the normal storage tissue with large starch grains. Such shallow penetration was found to be the rule in at least twenty suckers examined. While several different soils were used, in all good drainage, daily watering, and ample aeration were provided.¹

(b) *Deeper mass penetration of suckers.* Among the collection of suckers examined, a few showed a deeper mass penetration. The general details were similar to those already described, with the difference that

¹ With regard to cork formation over wounded surfaces there have, of course, been many investigations on storage organs such as the potato and sweet potato (*Ipomoea batatis*, Convolvulaceae). For example, Weimar and Harter, Journ. Agric. Res., xxi, 1921, showed that sweet potatoes develop a cork layer over wounded areas under moist conditions and a hard dry surface layer under dry conditions, the healed surface in both cases forming a fairly effective barrier against infection by micro-organisms. The formation of periderm was closely related to temperature, being most rapid at 33°C, and much less rapid at lower temperatures. Cork formation was most rapid at high humidities and was inhibited in dry air. A small degree of transpiration at the exposed surface is regarded as necessary for cork formation, while many writers regard oxygen supply as a determining factor. As in the banana sucker the formation of a cork layer is preceded by the formation of a layer of starch-free cells.

penetration of the ground tissue was more extensive, as shown in Fig. 33. The decayed tissue appears as a number of wedges in the healthy tissue, the apex of each coinciding as a rule with a diseased vascular strand. The

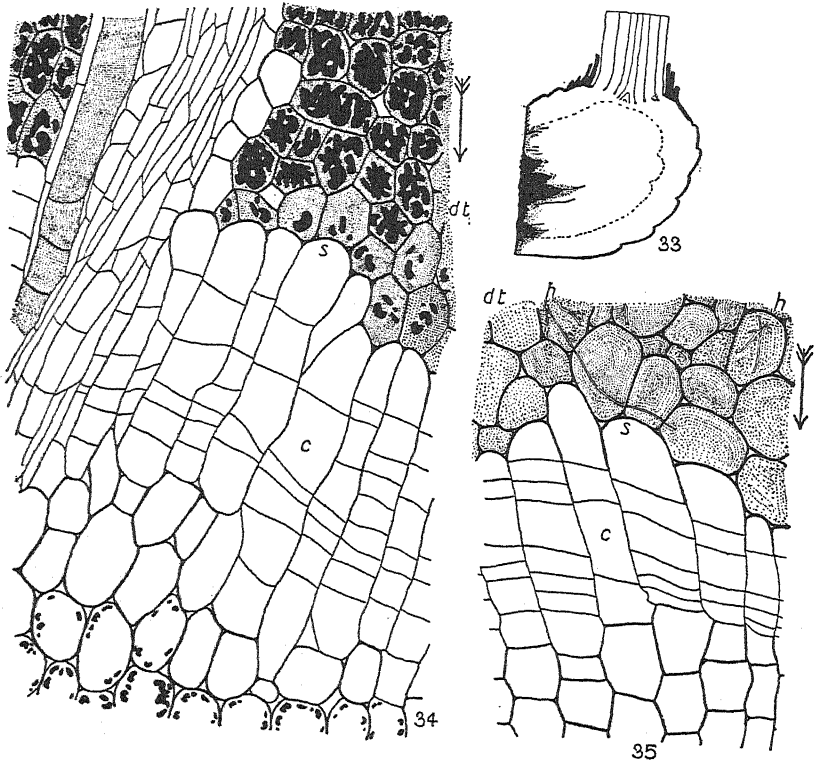


FIG. 33-35. Fig. 33. An example of deeper sucker penetration after 50 days in inoculated soil. The black indicates affected tissue, and stippling the cambiform barrier running right round it. $\times 2/5$. Fig. 34. A detailed study, longitudinal section, of the cambiform barrier shown in Fig. 33. *dt.* = dead tissue, killed in advance by diffusion of fungal secretions. *s.* = suberized walls of cambiform layer (*c*). $\times 100$. The arrow indicates the direction of diffusion and invasion. Fig. 35. A study of another part of the sucker in longitudinal section. Hyphae (*h*) in the dead tissue (*dt.*) have advanced right up to the cambiform barrier (*c*) with suberized walls (*s*) but no further killing or penetration have taken place. $\times 110$.

brown diseased tissue is surrounded on the inside by a clear hyaline layer—a continuous cambiform barrier—interrupted only by the longitudinally-directed vascular strands. Detailed studies are illustrated in Figs. 34 and 35. Sections taken from various parts of the sucker showed minor differences in detail. In Fig. 35, for example, the cells outside the cambiform layer have had all their starch removed, and have been invaded by hyphae. When companion sections were treated with concentrated sulphuric acid the cellulose walls were dissolved leaving behind a skeleton of suberin (Fig. 36). It will be seen that a complete layer of suberin has been

deposited in the outer walls of the cambiform layer, while one, and sometimes two, of the transverse partitions have also been impregnated. Some of the tissue outside the cambiform layer was also found to be completely or partially suberized. In his summary of the properties of suberized cells, Haberlandt (3) remarks:

‘Among other physiologically interesting features of corky walls, which are deserving of special mention, is the circumstance that pits are

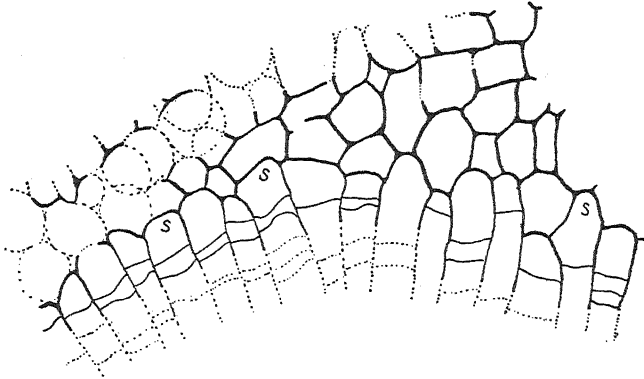


FIG. 36. A companion section to Fig. 34 treated with concentrated sulphuric acid, so as to leave the suberin skeleton (s). It will be seen that one and sometimes two of the transverse partitions of the cambiform layer have become suberized. $\times 50$.

generally absent. Where they do occur, they are, according to Von Hohnel, confined to the inner cellulose layer, and never penetrate into the suberin-lamella. These pits are most conspicuous where the cellulose layer is secondarily thickened, and are evidently functional only so long as the cork cells are alive and undergoing differentiation; they serve to facilitate the access of plastic materials to the developing suberin-lamella’.

(c) *Sloughing-off of decayed tissue.* A very young sucker, three-quarters of an inch in diameter, just emerging from the parent sucker, was cut out from the latter in the form of a small cube. The inner side of the cube was inoculated, and the sucker was then planted in a pot of good soil, watered regularly, and examined after sixty days. During this time the sucker had grown and produced a few leaves. When examined, it was found that all the tissue of the mother sucker had been completely exploited and sloughed off. Detailed examination showed that not only had a cambiform layer been formed at the cut inoculated end, but also that a similar barrier had developed all the way round where the young sucker had come into contact with the diseased parent tissue. The vascular strands of the young sucker were discoloured, red and yellow, and could be traced through the meristem into the young leaves.

A second young sucker, treated in the same way, also showed a

well-marked cambiform layer and only a few discoloured vascular strands. The diseased tissue of the parent sucker had again been sloughed off close to the suberized cambiform barrier.

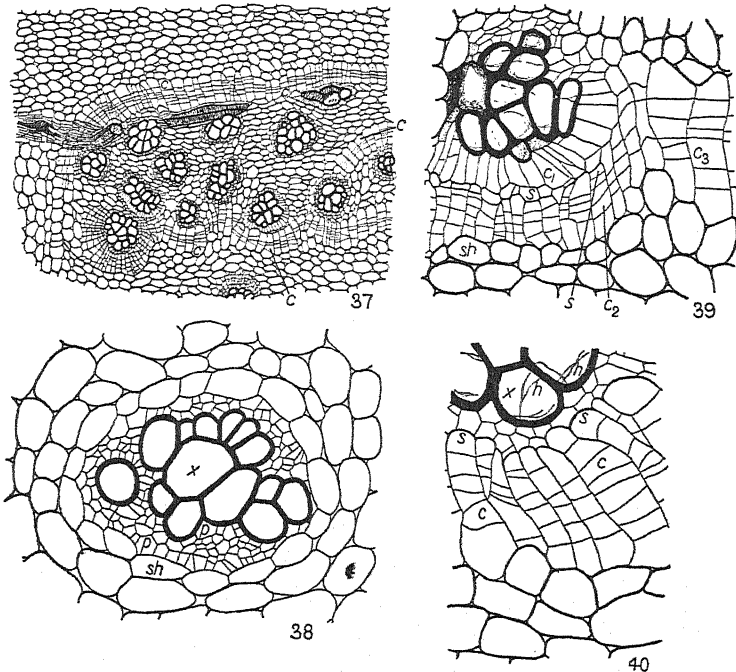
(d) *Injury to emerging young suckers.* At an early stage the young emerging sucker might be compared to the 'eye' of the potato. As *F. cubense* is a wound parasite, any injuries to young suckers or 'eyes' should afford points of entry. During the course of these observations cases of injured 'eyes' were noted. On detailed examination it was found that the outer tissue of the damaged meristem had developed a typical protective layer over the whole injured area. In one of the larger 'eyes', more severely damaged, the vascular strands were discoloured a short distance in.

(e) *Penetration into the vascular strands.* In its later stages, Panama Disease is over and above all significant as a vascular disease. This is true of most wilts caused by *Fusarium*. Once hyphae gain access to the wood-vessels they may traverse long distances unimpeded by transverse partitions. It is as a vascular parasite that *F. cubense* is usually recognized both in the sucker and in the aerial pseudo-stem. By inference it is suggested that the fungus is in its most favourable surroundings in the wood-vessels, and that, in attacking a root or sucker, invasion of the xylem might be regarded, speaking teleologically, as the objective. Whether this is the true interpretation or not remains to be seen. If recent theories regarding the paucity of foodstuffs in the wood-vessels are correct (5), one is led to speculate on the reasons why hyphae should leave well-stocked cortical cells for the scanty food supply in the conduits. It may also be mentioned at this point that the hyphae in the vessels are mostly unbranched and slender, suggestive of growth under adverse conditions. The following observations on diseased vascular strands throw additional light on the subject.

When suckers grown in pots of uniformly moist, well-aerated soils for eighty days were examined, it was found that mass penetration of the cut end was slight, while vascular infections extended inwards for 1 to 1.5 cm. at most. In the sucker, with typical monocotyledonous vascular arrangements, there is a preponderance of vascular strands at the periphery of the stele, and disease is usually most marked in this region. A detailed examination of such material in transverse and longitudinal sections brought to light structural and biochemical changes of considerable interest. It was found that as a result of the presence of hyphae in the wood-vessels a number of different growth activities had been induced in the adjoining parenchyma. Diffusion of fungal secretions in the direction of the endodermis led to the formation of cambium of pericyclic origin, while in adjacent areas of the ground parenchyma cambiform barriers had also developed. In Fig. 37, where a transverse section of a small area is shown

in some detail, three layers running in different directions may be observed. Growth reactions, however, were not confined to the ground tissue but were also observed in the parenchymatous cells of the vascular strands (Fig. 37), where many important structural changes had taken place.

At the periphery of the stele the vascular strands are somewhat con-



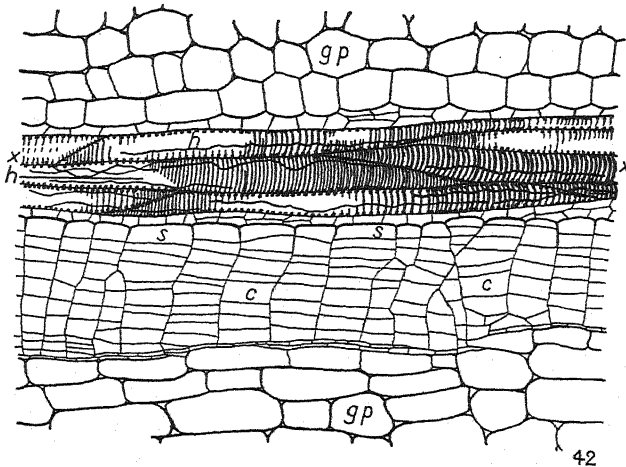
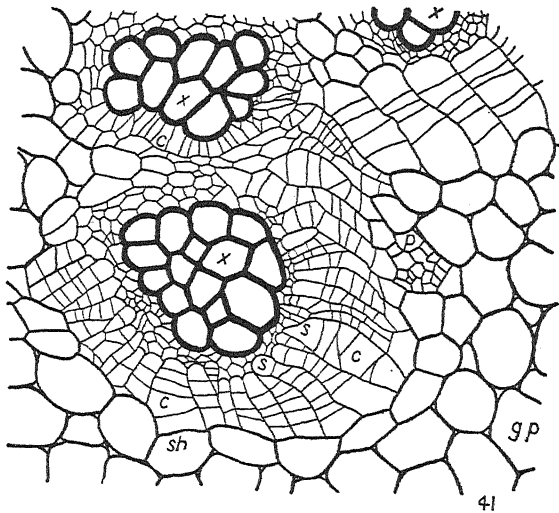
FIGS. 37-40. Fig. 37. Transverse section through the peripheral vascular strands (diseased) of an inoculated Gros Michel sucker. Cambiform formations (*c*) can be seen both in the ground parenchyma and in the vascular strands. $\times 20$. Fig. 38. A normal peripheral vascular strand in transverse section from a healthy sucker. *x* = xylem; *p* = parenchyma; *sh* = sheath cells. $\times 90$. Fig. 39. Transverse section through a diseased vascular strand, showing the infected vessels. *c*₁ = beginning of cambiform formation in cells abutting on the vessels; *c*₂ = older cambiform tissue derived from cells abutting on the sheath; *c*₃ = cambiform tissue formed from the ground parenchyma; *s* = suberized walls. $\times 90$. Fig. 40. Detailed study showing the suberized walls (*s*) of the cambiform tissue. *h* = hyphae in the xylem (*x*). $\times 130$.

densed and depart from the typical monocotyledonous arrangement. The xylem is more or less homogeneous, sometimes with peripherally disposed protoxylem elements, and is surrounded and partially invaded by thin-walled parenchyma in which the rather ill-defined phloem elements are present. The small-celled tissue is encased within a sheath of larger cells. Outside lies the typical large-celled, starch-containing ground parenchyma. In Fig. 38, the structure of a normal vascular strand is shown in detail. In transverse sections of diseased strands a very different state of affairs was found, and Figs. 39, 40, and 41 show that cambiform layers may develop

from any of the living cells. The vascular 'cambium' was sometimes formed from small cells abutting on the wood-vessels, sometimes from those abutting on the sheath cells, and sometimes from intermediate layers. The reactive power of these cells is very considerable indeed. In Fig. 39, the cells abutting on the wood-vessels (in which hyphae can be discerned) have commenced to elongate, prior to transverse septation. Just outside, other parenchymatous cells can be seen in their original state. Further out again, however, the stelar parenchyma abutting on the large sheath-cells has also been stimulated to form a well-defined cambiform tissue. It will be seen that by the elongation and division of one of the sheath cells this layer is in continuity with cambiform tissue formed from the larger cells of the ground parenchyma. It will further be noticed that these layers lie at right-angles to the direction of diffusion of toxic substances, thus bearing out what has already been frequently observed. Fig. 41 shows the considerable changes that have taken place in three adjacent vascular strands and in the adjoining ground parenchyma. In the topmost vascular strand the first changes in the parenchyma are just beginning to take place, but in the lower strand a well-defined cambiform layer has already developed. In the top strand, cells abutting on the xylem will be responsible for the formation of cambiform tissue, while in the lower strand the cells adjoining the xylem are unchanged (structurally), but cells adjoining the sheath have expanded and divided. At the right side of this strand it will be seen that a little pocket of parenchyma has been considerably displaced by the expansion of cells lying within. To the extreme right, the large-celled cambiform tissue has originated from modified sheath cells. In such sections hyphae and gummy substances could be observed in the wood-vessels; the latter were frequently found to be in a state of partial disintegration and collapse (Fig. 39).

In previous Sections it has been seen that the formation of a cambiform tissue is always accompanied by the suberization of walls on the side towards the infection. Detailed examination of modified vascular strands likewise showed that the walls of the cambiform layer adjacent to the infected tissue, i. e. wood-vessels were strongly suberized. This is shown in Fig. 40, where the well-defined suberized walls can be seen on the innermost limit of the cambiform barrier. We thus arrive at an interesting contrast. In the superficially infected root, the structural and biochemical changes which take place keep the fungus out of the vascular strands, while in the sucker, where hyphal penetration of the vascular strands has been effected through the cut ends, comparable changes are made by which the fungus and its toxic secretions are confined within the wood-vessels. These observations also contribute towards explaining why the fungus does not escape from the wood-vessels. The presence of completely suberized walls undoubtedly prevents the toxic solutions from diffusing

into the ground parenchyma where a more severe parasitism would inevitably result. The importance of the physiological condition of the sucker



FIGS. 41 and 42. Fig. 41. Cambiform formations (*c*) round diseased vessels (*x*). *p*. = pocket of vascular parenchyma isolated by cambiform activity; *g.p.* = ground parenchyma; *sh.* = sheath; suberized walls. $\times 90$. Fig. 42. Longitudinal section through a diseased strand. *x*. = xylem with hyphae (*h*); *c*. = cambiform tissue with suberized walls (*s*) towards the vessels; *g.p.* = ground parenchyma. $\times 90$.

tissues, and consequently their reactive powers, is further indicated by such observations. The relation of the suberized cambiform tissue to infected wood-vessels is shown in longitudinal section in Fig. 42. As indicated by some of the transverse sections, cambiform tissue may develop as one side

only, presumably depending on space and mechanical factors. One, two, and occasionally three slightly branched, hyphae can be seen in the wood-vessels, but not in any of the other tissues of the vascular strand, thus con-

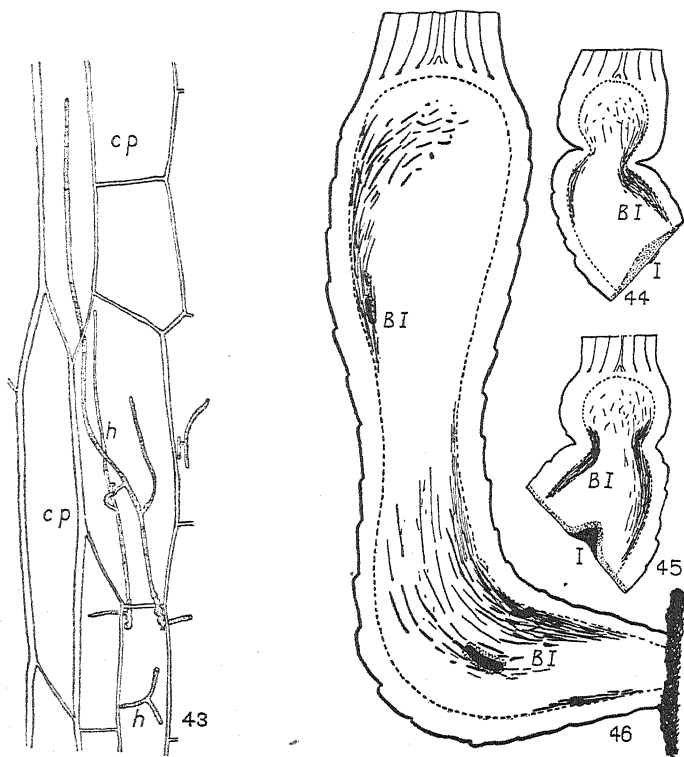


FIG. 43. Hyphae of *F. cubense* (h) in the conjunctive parenchyma, c.p. $\times 370$.

FIGS. 44-46. Figs. 44 and 45. Suckers of Gros Michel inoculated at (I) with *F. cubense* and grown for 80 days in moist well-aerated soil. Only a slight penetration, held in check by a cambiform formation, has taken place. The major vascular infection (B.I.) depicted was due to *F. cubense* following an attack by weevil borer. $\times \frac{1}{2}$. Fig. 46. Surinam Gros Michel banana, attacked at two separate points by weevil-borer and *F. cubense*. B.I. = borer infection. The black lines indicate the discoloured infected vascular strands. It will be noted that there is no infection at the point of junction with the parent sucker. $\times \frac{1}{2}$.

firming the observations of previous investigators. Lower down, in the region of mass infection, however, hyphae may be found in all the vascular tissues. An example is shown in Fig. 43, where they are traversing the elongated cells of the conjunctive tissue.

VII. INFECTION OF GROSS MICHEL SUCKERS IN PRESENCE OF WEEVIL-BORER (*COSMOPOLITES SORDIDUS*).

A collection of twelve healthy young Gros Michel suckers had been inoculated on the cut basal end with *F. cubense*, and planted in three different soils, one acid, one alkaline, and the third a typical potting com-

post. In all three soil types, some of the plants after eighty days showed preliminary Panama Disease wilt symptoms, while others remained healthy and had good foliage colour. On examination all the healthy suckers showed only the typical shallow penetration at the inoculated end, and the usual protective devices were in evidence.

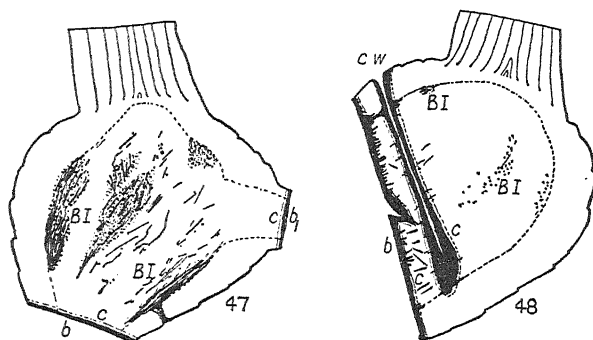
When the diseased suckers were examined, however, it was found (Figs. 44 and 45) that the red and yellow vascular strands, characteristic of Panama Disease, had not become infected by way of the inoculated basal surface. As before, the latter showed shallow penetration only, and cambiform layers were again in evidence. The vascular infections had originated at the neck of the sucker, about soil level, and from there had proceeded upwards to the meristem and leaves and downwards towards the basal end. Each of the diseased suckers had been invaded by weevil-borer (*Cosmopolites sordidus*), an organism not only prevalent in the West Indian Islands, but with a wide distribution in the Tropics. Larval burrows could be traced from the outside inwards, and the disposition of tissue affected by *F. cubense* coincided with the extent of the burrowing. This circumstantial evidence suggests that in these suckers the hyphae of *F. cubense* had followed the paths of the larvae. The damage caused by the latter to vascular strands and ground tissue, together with the considerable quantities of food materials made readily available for the hyphae, would create a zone of mass decay, from which an extensive amount of vascular infection might well result.¹

Observations made on diseased Surinam Gros Michel bananas growing on an infected College plot brought to light the following points. Young suckers were found to have Panama Disease infections which coincided with the paths of the weevil-borer larvae (Fig. 46). One of the suckers (produced from a deep-seated parent sucker) had two separate fungal infections along its length, each of which was associated with a separate larval burrow. No basal infection had taken place.

In another pot experiment four plants after 115 days showed characteristic external symptoms of wilt. The cut ends were again found to be completely protected, so that vascular infection had not originated from this source. Varying amounts of vascular discoloration were present in the middle and upper portions of all four suckers. Again, this was definitely associated with larval burrows, the infections originating laterally near the neck and progressing upwards and downwards (Figs. 47 and 48). In Fig. 47 the two cut surfaces exposed to the infected soil, namely, the basal

¹ Leach (Phytopath, xvi, 1926) found a somewhat similar association between 'Black-leg' of potato, and the seed-corn maggot (*Phorbia fusciceps*). The insect deposits its eggs, which are superficially contaminated with the pathogenic bacteria, in the soil near the healthy seed-pieces or sprouts. The larvae inoculate the seed-pieces with bacteria and aid the development of the disease by destroying or preventing the formation of wound cork.

end and the point of separation of a small sucker are both protected, the extensive infection being again of lateral origin. In Fig. 48, where the infection was slight, the sucker had not only the basal end exposed to infection, but a large cutlass wound afforded an additional point of entry. All the cut surfaces, however, had developed protective barriers, and



FIGS. 47 and 48. Suckers of Gros Michel with borer infections. *b.* = basal cut surface; *b₁* = cut surface at point of separation of young sucker; *c.* = cambiform barrier; B.I. = borer infection; *c.w.* cutlass wound. $\times 1/3$.

although the vascular discoloration there was slightly more marked than before, the infection in the body of the sucker was of other origin.

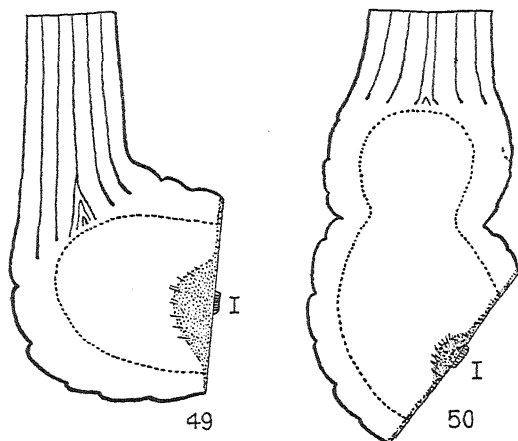
All varieties of bananas are subject to attack by weevil-borer. When the Gros Michel is attacked, with *F. cubense* present, effective parasitism by the fungal organism has frequently been found to coincide with the larval burrow. When other varieties, such as the Lacatan and Canary, are attacked under the same conditions, the typical vascular infection does not result. While the evidence submitted here is admittedly purely circumstantial, the parasitism of *F. cubense* in the presence of weevil-borer appears to merit careful attention.

VIII. INFECTIONS OF GROS MICHEL SUCKERS IN CLOSED MOIST CHAMBERS AND IN WELL-AERATED SOILS.

Fifteen small Gros Michel suckers of even size were inoculated in the centre of the cut basal end with *F. cubense*. Three were placed in a closed moist chamber, and the remainder were planted in three boxes of well-aerated, uniformly watered soils.

The three suckers in the closed chamber were examined after nine days. As the suckers were young, penetration was rapid. Fig. 49 shows the deepest penetration obtained. The planted suckers were examined after eighty-two days. Fig. 50 shows the deepest amount of penetration obtained in any of the suckers. Shallow penetration was the rule throughout, and detailed examination showed that well-defined protective layers

were in evidence. The amount of vascular discoloration was unimportant. The comparison suggests that carbon dioxide vitiation is a factor favouring effective penetration of the sucker.¹ This suggestion is also supported by an examination of the soils in which disease has been prevalent (10).



FIGS. 49 and 50. Two contrasted infections of Gros Michel suckers. Fig. 49. After 9 days in closed moist chamber. Deep mass infection. Fig. 50. After 82 days in moist, well-aerated soil, showing shallow mass infection and cambiform barrier. $\times \frac{1}{2}$.

Another experiment bearing on this subject was carried out as follows: Four disease-free suckers of comparable age and size were inoculated with spores at the cut basal end. Two of the suckers were then covered up with a layer of puddled clay, i. e. clay kneaded in a moist condition so as to exclude as much air as possible and thereby cut down interchange of gases. The puddled suckers were then set in pots of compost. The two remaining suckers were placed directly into compost to serve as controls. When examined in longitudinal section after sixty-seven days the unpuddled controls showed the typical shallow penetration only, and vascular infection was also limited. The foliage and roots of both control and puddled suckers were good. When the latter were cut in longitudinal section, a mass penetration, 1 cm. deep, over the whole basal

¹ In an extensive work dealing with the infection of potatoes by species of *Fusarium*, Schmidt (Arb. Biol. Reichsanstalt für Landw- und Forstwirtschaft, xv, 1928) has shown that the degree of infection varies according to the species of *Fusarium* used, temperature and humidity. *F. coeruleum* will cause infection at an atmospheric humidity of 50 per cent., while *F. viticola* and *F. avenaceum* require at least 80 per cent. Once a tuber is infected by *F. coeruleum* he states that rotting progresses slowly even under conditions detrimental to the fungus, whereas in similar cases the development of *F. avenaceum* and *F. viticola* is impeded by the suberization of adjacent tissues. The species under discussion are, like *F. cubense*, typical wound parasites. A correlation between variation in susceptibility to fusarial infection and fluctuating sugar content is suggested. In closed glass vessels a number of species, normally saprophytic caused rotting of some of the tubers, the conditions to which the latter were exposed being comparable to those obtaining in badly-ventilated storage rooms or slimy soils.

end was found (Fig. 51). This dark-coloured infected region was found to consist of the following, proceeding from the outside inwards: (*a*) zone of disintegrated tissue; (*b*) dark coloured zone with cells full of hyphae; (*c*) a sharp transition from the latter to pink-stained tissue. (*a*), (*b*), and (*c*) were without starch; (*d*) beyond the pink zone there was a transition to the normal starch containing tissue.

The reactions of the plant to fungal invasion under these growth conditions included starch removal and the tardy suberization of walls. When sections were dissolved in concentrated sulphuric acid, there was evidence that more than one layer had been incompletely suberized. There was no indication of a cambiform tissue. Little trace of vascular infection was present at this stage, but (as indicated by the stippling in Fig. 51) a slight discoloration pervaded a considerable part of the ground tissue of the sucker. It should be pointed out also, that while the sucker itself was closely enveloped in puddled clay, it had an abundant root system growing in the outer compost.

IX. LATERAL INFECTION OF GROS MICHEL SUCKERS.

When a sucker is planted there are, in addition to the basal cut end, two other possible infection courts. These are (*a*) the leaf-bases of old leaves, and (*b*) root-bases.

(*a*) Suckers growing in pots of highly infected soil were used for examination. When a moribund leaf breaks off, it does so close to the sucker. The outside of every sucker has thus a slightly corrugated appearance, due to the successive production and removal of leaves. A young exposed leaf-base, near the neck of the sucker, is shown in longitudinal section in Fig. 52. The point of separation is close to the body of the sucker. The exposed surface is of dark colour, and has associated fungi, but invasion is checked by suberization of the cell-walls at some distance in. The leaf-base is marked by a semicircular pocket of very small cells. Older leaf-bases, from the lower part of the sucker, were next examined in longitudinal section. It was found that the leaf-base was considerably decayed, as also was the adjacent tissue, and hyphae of various fungi were observed in the outer cells. No effective penetration had taken place, however, by reason of the presence of a single, and at some points a double, well-developed suberized cambiform layer (Fig. 53). The latter could easily be distinguished by the naked eye as a translucent layer, lying between the normal white storage tissue and the brown outer decayed tissue. These observations indicate that leaf-bases, like the cut basal end of the sucker, are adequately protected against fungal penetration.

(*b*) At the time of planting all roots are, as a rule, trimmed away from

the sucker. Each root-base thus presents a wounded open surface,⁷ and, theoretically, invasion of the sucker through the vessels of the root should take place rapidly. Observations conducted on a large collection of

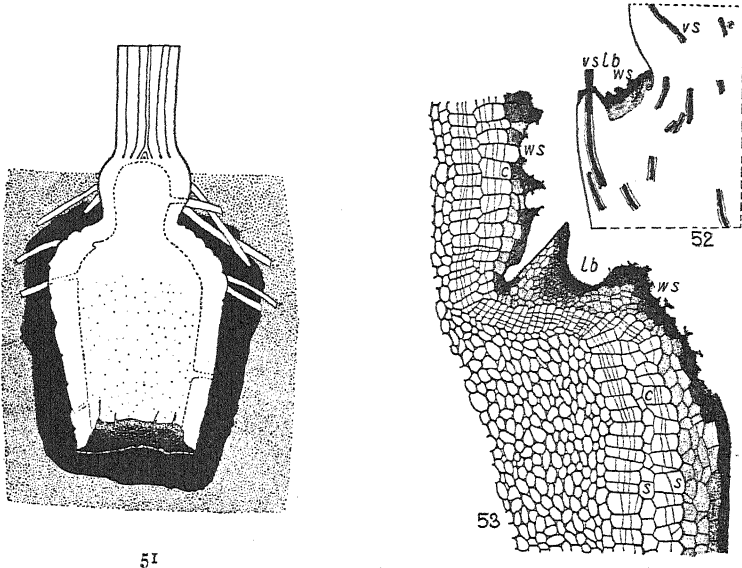
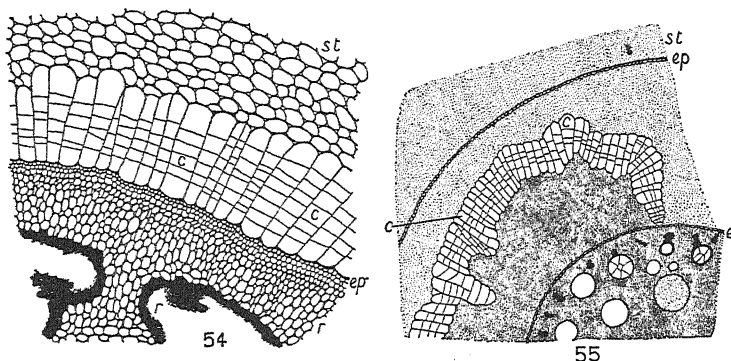


FIG. 51. Inoculated Gros Michel sucker which has been puddled round with clay (black) and potted in compost (stippled). The amount of penetration after 67 days is shown. No cambiform layer was present. $\times \frac{1}{3}$.

FIGS. 52 and 53. Fig. 52. Gros Michel. Longitudinal section through young leaf base. v.s. = vascular strand; w.s. = wound surface; l.b. = leaf base. $\times 5$. Fig. 53. Old leaf base. . = cambiform tissue. The dark and stippled cells are dead. $\times 20$; s. = suberized walls.

suckers growing in inoculated soils, however, have shown that infection only occasionally takes place through these points of entry. In Part I of this work the probable importance of tyloses in the vessels has been indicated. Since then the detailed study of root-bases, from suckers growing in highly infected soil, has been continued. On most occasions it was observed that the root-base was rotted for a short distance in, after which there was no further penetration. There were thus strong indications that some effective protective mechanism was in operation. It has already been seen that unspecialized tissues may become structurally modified and suberized so as to prevent the passage of hyphae. The difficulty to be overcome in protecting the root-bases lies in the blocking of the wide wood-vessels. For purposes of examination it was found convenient to make a series of tangential sections through the sucker from the outside inwards. In this way a series of transverse sections of the embedded root-base was obtained. At the outside of the sucker, and inwards for a distance of from 0.25 to 0.5 cm., the root was found to be decayed, disintegrated, and exploited by bacteria and the

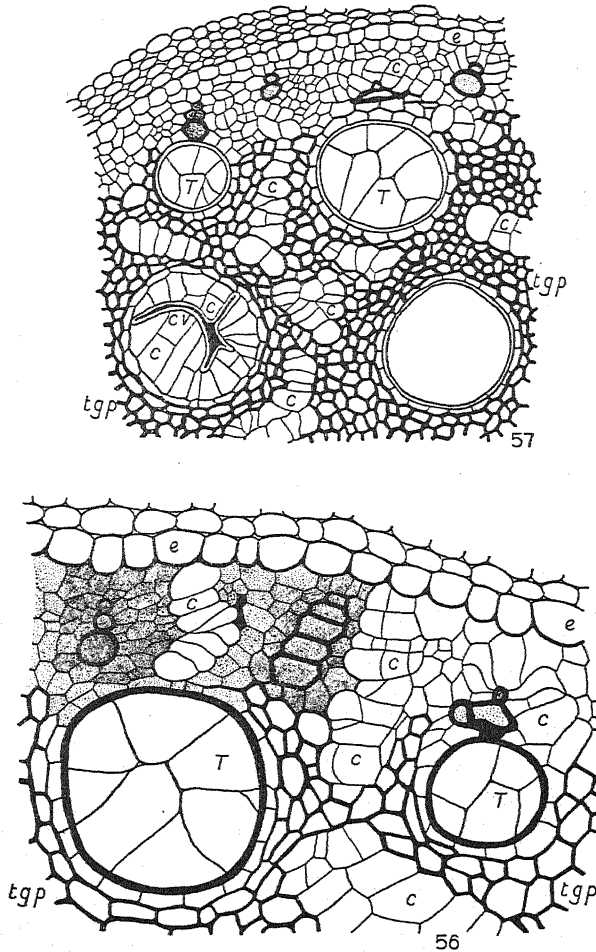
hyphae of several fungi. Further in the root tissue, though diseased, was coherent. At this point, the spread of infection from the root-base into the adjacent sucker tissue was prevented by the development in the latter



FIGS. 54 and 55. Fig. 54. Diseased Gros Michel root base embedded in the cortex of the sucker. *st.* = sucker tissue; *c.* = cambiform layer formed by the sucker tissue; *ep.* = epidermis; *r.* = diseased root cortex partly disintegrated. $\times 33$. Fig. 55. The same root at a point further in; the stele and part of the cortex are diseased—dark stippling. The outer cortex and the sucker tissue are disease-free—light stippling. The protoxylem is necrosed, and metaxylem vessels are full of gum (stippled vessel) or tylosed. *e.* = endodermis; *c.* = cambiform barrier; *ep.* = epidermis; *st.* = sucker tissue. $\times 22$.

of a deep suberized cambiform layer (Fig. 54). On proceeding inwards the amount of disease in the root cortex gradually decreases. It has been seen in Part I that infection proceeds along the stele much more rapidly than through the cortex. Further in, therefore, the toxic secretions would tend to diffuse out from the stele into the healthy cortical tissue, but this is controlled by the formation of suberized cambiform barriers in the cortex (Fig. 55). Infection of the sucker through the root cortex is thus prevented. In the outer necrosed region of the root-base all the tissues are penetrated by hyphae. Further in the brown necrotic appearance is localized in the parenchymatous tissue of the stele, while vessels may still show evidence of hyphae. It is at this point that reactions to fungal invasion in the stelar tissues begin to be apparent. Fig. 56 indicates some of the changes observed. Fungal secretions, localized in wood-vessels where hyphae are present, tend to diffuse into the adjacent parenchyma. This, however, does not take place unimpeded as indicated by the characteristic expansion, division, and suberization of cells. On the right-hand side, the conjunctive parenchyma, adjacent to infected protoxylem, has been stimulated to expand. The same section shows vessels which have been blocked by tyloses. As these are living cells, capable of becoming suberized, they provide a means by which penetration through the vessels may be hindered. The importance of tyloses in comparable circumstances is well known in other plants. Again, some of the large vessels were observed to

be full of gum, but as embedded hyphae could be discerned it is not known to what extent it acts as an impediment to fungal penetration. In addition to tyloses, another mechanism for preventing the passage of hyphae



FIGS. 56 and 57. Fig. 56. Stele of Gros Michel root base. Stippled tissue is diseased. T. = vessels with tyloses; c. = cambiform cells; e. endodermis; tgp. = thick-walled ground parenchyma. $\times 210$. Fig. 57. Another section from the same root. cv. = collapsed vessel; t. = vessels with tyloses; c. = cambiform cells; e. = endodermis. $\times 90$.

through the protoxylem and metaxylem vessels was observed. This consists of a more or less complete collapse of vessels induced by the expansion of the surrounding parenchymatous cells (Fig. 58). In such material softening of the lignified vessel-walls appears to have taken place. Figs. 59 and 60 show the way in which the conducting channel of the protoxylem is almost completely closed by such cell enlargement.

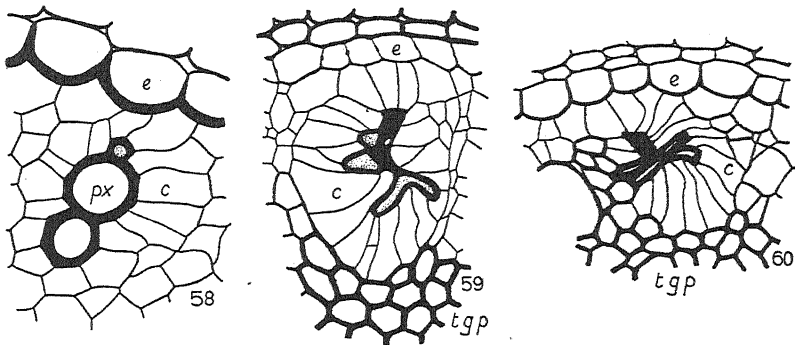
The same process takes place on a larger scale with the wider metaxylem vessels. Although the ground tissue in which the vessels are embedded may be partially indurated and thickened, the vessels themselves always remain surrounded by a jacket of thin-walled living cells. Fungal stimulation leads to an enlargement of these unspecialized cells. As they begin to expand, the lignified walls of the wood-vessels are pushed inwards (Fig. 61). This continues till the walls are brought close together (Fig. 62) and finally they are closely adpressed (Fig. 63) as the result of the considerable expansion of the parenchyma. Probably the rigid indurated ground tissue aids in the collapse of the vessels. A last stage is reached when the expanding cells divide by characteristic transverse walls (Fig. 64). Deep-seated sections, then, taken near the inner limit of root-base infection showed vessels which were either free from infection, filled with tyloses, or collapsed, as described above. Fig. 57 shows in detail a collapsed vessel and one blocked by tyloses in the same transverse section. There is no difficulty in distinguishing the two, as the thick xylem wall is always pushed in where collapse has taken place.¹

Such reactions are not confined to the roots of the Gros Michel alone, nor is *F. cubense* the only fungus that may be involved. A mass of data has been collected with regard to both the Lacatan and Canary varieties, but an example of each will suffice for the present purpose.

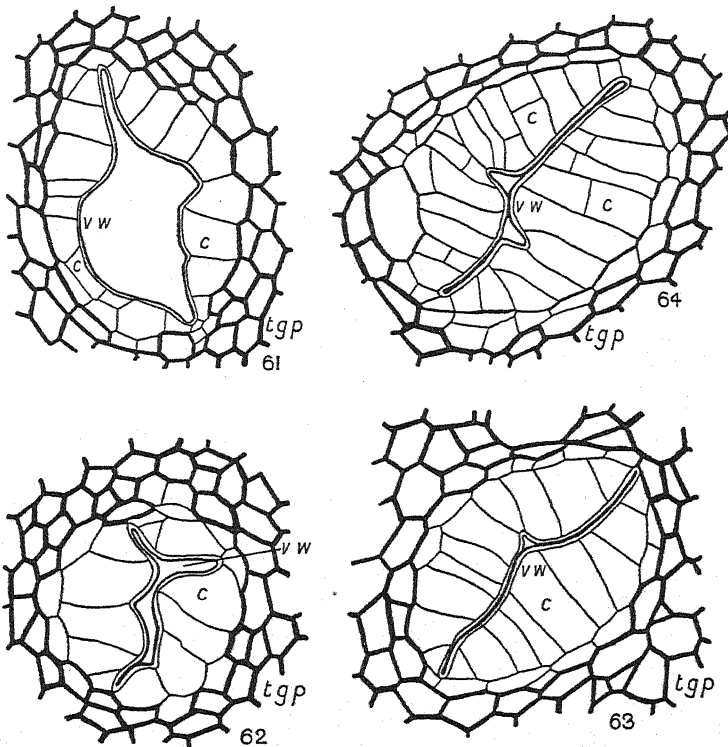
A root of the Lacatan banana with a vascular infection showed hyphae of a fungus (unidentified) in the vessels. In the region of maximum infection both cortex and stele were brown in colour, dead, and invaded by hyphae. Further back, however, only the stele was infected, and on proceeding inwards this gradually diminished. Detailed examination at one point showed that all the stele was penetrated by hyphae and their secretions except the pericycle. This had commenced to enlarge and divide in the usual way and had become suberized. Further back, where the infection began to taper out, only the more central metaxylem vessels and ground tissues were affected (Fig. 65). The latter tissue was brown and necrosed. Lethal effects did not extend to the endodermis, however, but ceased inside the protoxylem, where a suberized cambiform barrier had been formed from the cells of the ground parenchyma. This section also shows another interesting feature. The cells abutting on one of the infected vessels had been stimulated to develop, and had grown right into the vessel cavity. Some divisions by transverse walls had also taken place.

A somewhat similar condition was found in a diseased root of the Canary banana (Fig. 66). Here, a suberized cambiform barrier has preserved

¹ It has been shown by Gardner (Phytopath, xv, 1925) that the necrotic regions in tomato fruits affected by a certain type of mosaic are frequently characterized by proliferation of the adjacent cells. Various growth effects are recorded, including some which lead to a collapse of the vessels. The final results are not unlike those recorded here.



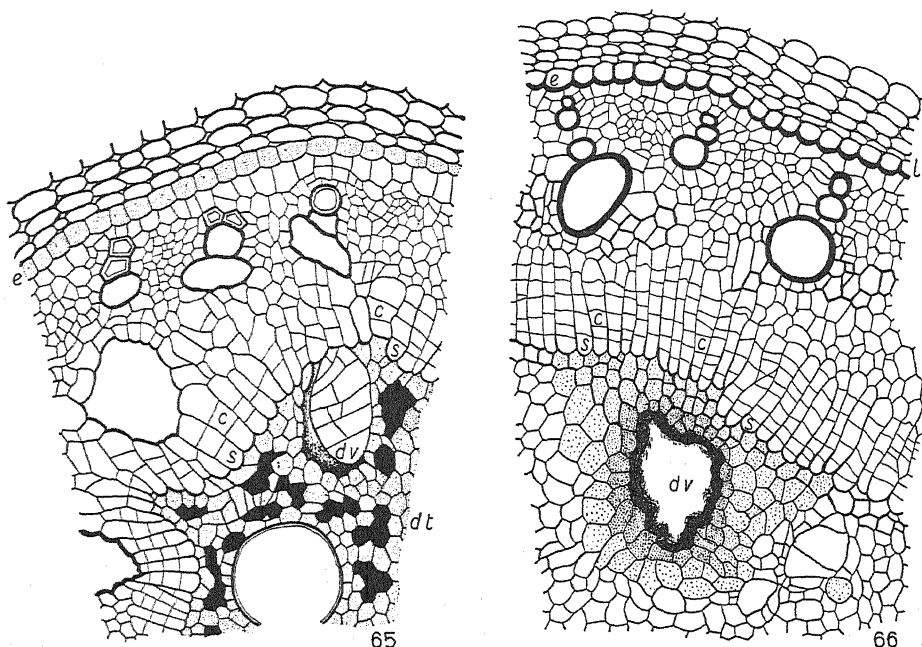
FIGS. 58, 59, and 60. Three stages showing the collapse of diseased protoxylem vessels. Fig. 58. *c.* = cells of conjunctive parenchyma stimulated to enlargement by the presence of fungal secretions. *px.* = protoxylem vessels; *e.* = endodermis. $\times 280$. Figs. 59 and 60. Collapse of protoxylem vessels by expansion of conjunctive parenchyma (*c.*). *t.g.p.* = thick-walled ground parenchyma. $\times 130$.



FIGS. 61, 62, 63, and 64. Four stages in the collapse of metaxylem vessels by the enlargement of the thin-walled cells of the conjunctive parenchyma into cambiform cells. *vw.* = vessel-wall; *c.* = conjunctive parenchyma which develops into cambiform tissue; *t.g.p.* = thick-walled ground parenchyma. $\times 210$.

the outer stelar tissues from the lethal solutions diffusing from a diseased vessel (fungus not identified), in the centre of the stele.

The accumulated evidence suggests that such reactions are characteristic of the genus *Musa* as a whole.



FIGS. 65 and 66. Fig. 65. Transverse section of Lacatan root stele; the central region of the stele is diseased, *dt.*, but a cambiform barrier (*c*) formed from the ground parenchyma is preventing the outward diffusion of toxic solutions. *dv.* = diseased vessel into which enlarging parenchyma cells have grown; *e.* = endodermis; *dt.* = diseased tissue; *s.* = suberized walls: black cells are necrosed. $\times 210$. Fig. 66. A comparable study of a diseased root of the Canary banana. *dv.* = diseased vessel from which toxic solutions are diffusing; *c.* = cambiform barrier formed from ground parenchyma; *e.* = endodermis, diseased cells are stippled; *s.* = suberized walls. $\times 140$.

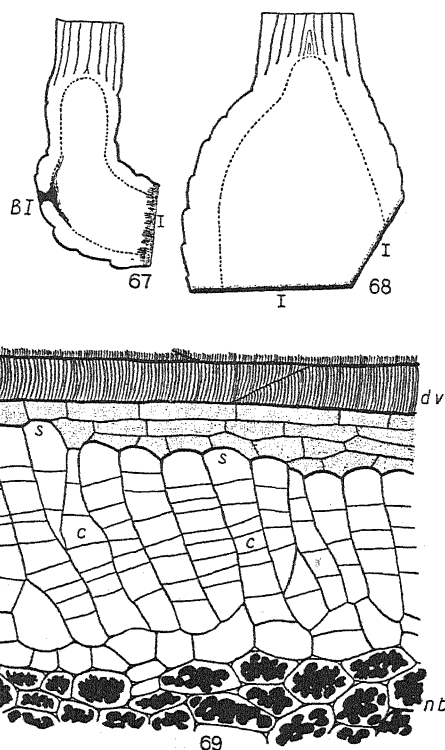
X. COMPARATIVE OBSERVATIONS ON DISEASE IN GROS MICHEL, CANARY, AND LACATAN SUCKERS.

Six suckers each of the Gros Michel, Canary, and Lacatan varieties were inoculated at the cut basal end, and, where possible, on the surface exposed by cutting off young suckers. They were then planted in 10-inch pots of compost, and examined after 54–61 days, having been maintained under uniform conditions during that time.

In the Gros Michel suckers the results were similar to those described in previous sections.

The Canary suckers were very similar (Figs. 67 and 68), with the difference that there appeared to be less development of cambiform tissue and more pronounced suberization. In a small sucker (Fig. 67), a lateral borer infection, accompanied by a certain amount of vascular discoloration

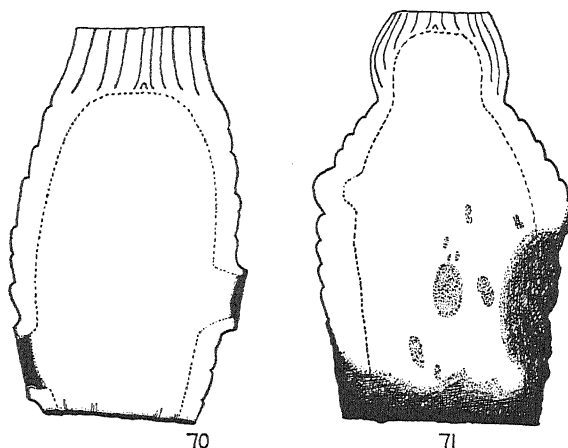
(brownish red) was observed. In longitudinal sections through the infected vascular strands reactions similar to those described for the Gros Michel were observed, further penetration into the cortex being again prevented by the presence of a strongly suberized cambiform layer (Fig. 69).



FIGS. 67-69. Figs. 67 and 68. Suckers of the Canary (Governor) banana exposed to *F. cubense* in moist soil for 54-61 days—shallow penetration only. Fig. 67 shows a slight borer infection, B.I.; I. = inoculated basal end. $\times \frac{1}{3}$. Fig. 69. Longitudinal section through tissue affected by borer and fungus. A suberized cambiform tissue prevents diffusion of toxic solutions from the diseased vessels. *dv.* = diseased vessels; *c.* = cambiform tissue; *s.* = suberized walls; *nt.* = normal storage tissue with starch grains. $\times 60$.

The Lacatan suckers also showed limited penetration at the cut surfaces. Fig. 70 shows a large sucker inoculated at the cut basal end, and also at four other exposed surfaces where young suckers had been cut off. Penetration into the latter was deeper than into the adult tissues at the basal end. Megascopically a pinkish colour marked the junction of affected and healthy tissue. At the basal end hyphal penetration had gone about six cells deep, the affected cells being of a dark brown colour. Further invasion was held in check by the presence of a suberized cambiform layer. The latter, however, was feebly developed, and had undergone single transverse divisions only. Treatment of sections with concentrated sulphuric

acid left behind a well-developed suberin skeleton (Fig. 73). The inoculated surfaces where young suckers had been cut off showed no indication of a cambiform tissue. Two distinct zones were present: (a) an outer invaded zone with profuse hyphae; the cell walls were brown in colour and un-suberized; (b) an uninvaded zone where the cell-walls were of a pinkish

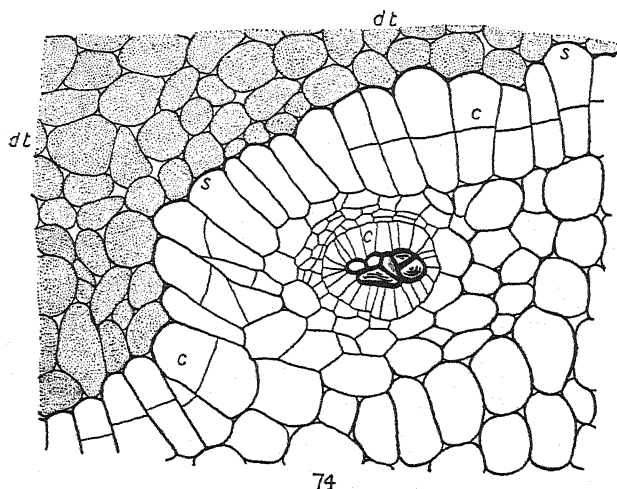
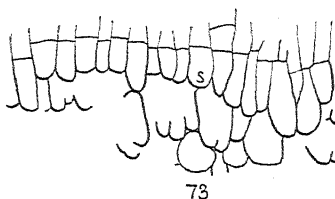
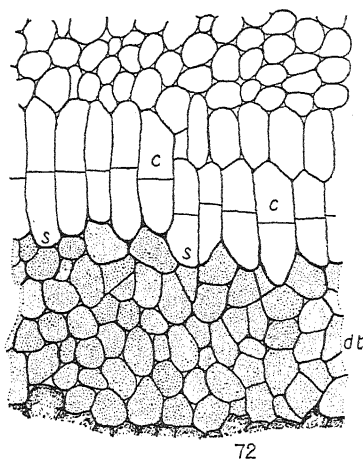


FIGS. 70-71. Lacatan suckers exposed to *F. cubense* in well-aerated moist soil for 54-61 days. Fig. 70 shows slight basal infection and deeper infection at the lateral cut surfaces. Fig. 71, sucker which has been attacked by weevil-borer and other animal agencies. $\times \frac{1}{2}$.

colour, and partially or completely suberized. A layer of ten or more cells was found to resist solution on treatment with sulphuric acid. As unsterilized soils were used, soil organisms other than *F. cubense* may, of course, be present in the decayed tissue.

One of the Lacatan suckers was found to be badly affected by weevil-borer (Fig. 71); the result was a considerable basal and lateral zone of decay together with channels where penetration was in progress. The decayed tissue was moist, soft, and black, and freely invaded by bacteria and various fungi. The infection, however, had not extended into the vascular strands. Diffusion of toxic substances and hyphal penetration at the basal end were checked by the suberization of walls, but no cambiform tissue was present. In the upper region of the lateral infection, however, a cambiform tissue was observed. Examination of the distal end of a borer tunnel showed that the brown-coloured decayed tissue was also surrounded by a suberized cambiform barrier (Fig. 74). When vascular strands passing out from the diseased into healthy tissue were examined in transverse section, it was found that solutions drawn off from the former had stimulated the vascular parenchyma. In Fig. 74 a vascular strand is seen in the healthy tissue just outside the cambiform layer; the vascular parenchyma has expanded, and the vessels are showing signs of collapse. All stages in vessel collapse as the result of such changes in the

adjoining parenchyma were observed. A comparison of the suckers of the three varieties indicates that, with minor differences, the same general mechanisms operate to prevent invasion by soil organisms at exposed cut surfaces.



FIGS. 72, 73, and 74. Fig. 72. Longitudinal section through infected basal region of sucker in Fig. 70. *dt.* = diseased tissue; *c.* = cambiform layer with suberized walls (*s.*). $\times 50$. Fig. 73. Suberin skeleton, *s.*, left behind after a companion section to Fig. 72 was treated with concentrated sulphuric acid. $\times 50$. Fig. 74. Section through junction of diseased and healthy tissue in Lacatan sucker affected by weevil-borer and fungus. *dt.* = diseased tissue; *c.* = cambiform layer; *s.* = suberized walls. Note also the infected vascular strand in the healthy region of the cortex. The expansion of the conjunctive parenchyma (*c.*) is causing partial collapse of the vessels. $\times 90$.

XI. DISCUSSION OF RESULTS.

In this paper a statement of facts has been submitted regarding the early stages of infection of the Gros Michel sucker by *F. cubense*. The nature of a disease which normally may take a year to run its course in the field cannot be prejudged on observations limited to the first few months of exposure to the parasitic organism. In previous memoirs the initial stages of infection have not been considered in much detail. The object of the present work was to obtain information on this subject, and as the result of detailed examination of early stages of infection new and interesting facts have accrued. Collectively these show that despite the virulent parasitism attributed to *F. cubense*, inoculated suckers grown under conditions of uniform moisture and adequate aeration, prevent penetration by hyphae, and also diffusion of toxic substances by the formation of well-developed suberized cambiform layers. Such growth reactions always bear a definite relation to the position of the penetrating organism, i. e. the cambiform layer is formed at right angles to the direction of diffusion of toxic secretions. During the first three months of attack, under the aforementioned conditions, not only is mass penetration shallow, but vascular infection is also insignificant. These observations are, of course, set down without prejudice to greater degrees of infection that may be found during later phases in the metabolism of the sucker and the life of the plant as a whole. The results obtained are simply records of the limited parasitism found when suckers are maintained under favourable soil conditions. The question of vascular infection and the frequently observed fact that the hyphae are limited to the wood vessels have been discussed in the text. In the light of observations recorded here this phenomenon may be discussed from a new point of view.

As the fungus kills in advance by the diffusion of its toxic secretions, the latter deserve careful consideration. In his paper of 1919 Brandes, in an interesting series of experiments, showed that the final wilting symptoms are not due to a plugging of the wood vessels, but to toxic substances secreted by the fungus. He demonstrated the wilting power of staled *F. cubense* solutions on buckwheat and bean seedlings, but not on the banana itself. In the present work it has been shown that sucker tissues react rapidly to the presence of fungal secretions by the removal of starch, suberization of walls and formation of cambiform layers. These reactions, which are present in the vascular system and ground tissue alike, occur also in relation to wounding.

As *F. cubense* is principally a wound parasite it is, on a *a priori* grounds, liable to gain entry into the sucker through any perforation caused by animal agency. Circumstantial evidence indicates that the entry of weevil-

borer accompanied by *F. cubense* may lead to the appearance of Panama Disease symptoms in the sucker.

In conclusion, the general evidence advanced here indicates that the intensity of infections and the power of the plant to react are conditioned by certain external factors. These are being held over for discussion in a later work. The importance of the observations recorded here rests in the fact that they indicate methods of research that will be profitable to follow, and at the same time afford a series of criteria by which the activities of invading parasitic organisms may be gauged.

XII. SUMMARY.

1. The purpose of the present paper has been to submit observations on the early stages of sucker infection by *F. cubense*.

2. Suckers inoculated in closed moist chambers show considerable amounts of mass and vascular infection after ten to twenty-five days. Young tissue is more deeply and rapidly penetrated than older tissue. The resistant or immune varieties may also show a certain amount of sucker penetration when inoculated under these conditions. This, however, was always less than that observed in the susceptible Gros Michel.

3. Anatomical investigation showed that after some time further penetration of *F. cubense* into the sucker was resisted by the formation of a suberized cambiform tissue in the ground parenchyma at right angles to the direction of invasion.

4. In the fungal penetration of the sucker, killing takes place in advance by the diffusion of toxic fungal secretions. Cells invaded by hyphae are found at a considerable distance behind the innermost point of killing.

5. By immersion of cut ends of suckers in staled culture solutions important protective tissue reactions were induced in the course of four days. These include starch removal, suberization of cell walls, and the formation of cambiform tissue.

6. Experiments carried out to determine the rate of penetration into growing suckers showed that suckers maintained in uniformly moist, well-aerated, soil for fifty to ninety days had a more shallow infection than that obtained in closed moist chambers in ten to twenty days. The former were provided with well-developed suberized cambiform layers along the exposed surfaces. Vascular infection under such conditions was also slight.

7. The question of vascular infection is considered, and evidence has been recorded which shows that, whereas in root infection structural changes take place which keep hyphae out of the wood vessels, in the sucker, once vascular infection has taken place, similar structural changes take place by which the hyphae are kept within the vessels.

8. Cambiform tissues of vascular origin are described in detail. It is shown that, in relation to the presence of hyphae in the vessels, suberization takes place on the innermost walls of the cambiform tissue, i.e. towards the xylem.

9. Observations on the inoculation of Gros Michel suckers in the presence of weevil-borer suggest the importance of the latter in bringing about infection by *F. cubense*.

10. Comparative inoculations of Gros Michel suckers in closed moist chambers and in uniformly moist, well-aerated soils indicate that carbon dioxide vitiation or inadequate aeration is an important factor in determining the extent of infection.

11. A preliminary account of fungal penetration into root-bases is given.

12. The general evidence submitted indicates that the extent of penetration of suckers is conditional.

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Notes on Conifers.

VII. *Pherosphaera Hookeriana* Archer.

BY

W. T. SAXTON.

With eight Figures in the Text.

FROM time to time doubts have been cast on the systematic position usually accorded to *Pherosphaera* as a member of the Podocarpaceae. The erect axillary ovule without an epimatium is certainly not typically Podocarpean, yet the erect axillary position is duplicated in *Phyllocladus*, and the epimatium is reduced to quite small proportions in *Podocarpus* and *Acmopyle*. A recent investigation of the life-history by Lawson (4) showed various features tending to exclude the genus from the Podocarp alliance, such as the absence of prothallial cells from the pollen grain, and the lateral position of the archegonia. On the other hand, the winged pollen is, externally, precisely like that of *Microcachrys*, and the solitary ovule per scale is also characteristic of Podocarpaceae. Lawson was led to the conclusion that 'in the gametophyte structures and embryo of *Pherosphaera* there are no features which justify our classifying this genus among the Podocarpaceae'. This statement was rather more sweeping than was justified by the facts, but nevertheless there seemed fairly good reason for regarding *Pherosphaera* as the type of a family separate from the Podocarpaceae. Apart from details of the life-history given by Lawson, *Pherosphaera* is a genus rather incompletely known. The vegetative anatomy of one of the two species was studied by Groom (2), but that of the other species, *P. Hookeriana*, has apparently not been examined. Moreover, there is no record, as far as the writer is aware, of the occurrence (or absence) of root nodules in either species. As such root tubercles are now known in every other genus belonging to the Podocarpaceae, i. e. *Podocarpus*, *Dacrydium*, *Saxegothaea*, *Microcachrys*, and *Phyllocladus*, as reported by Spratt (10), and *Acmopyle*, examined by Sahní (8), and have not been observed in any other Conifers, it seemed that it would go far towards solving the problem of the relationship of *Pherosphaera* to ascertain whether typical root tubercles are, in fact, present or not. Various attempts have been made to secure roots of either species, and the material for the present investigation

was received recently from Mr. F. T. Brooks, to whom it was sent by Mr. W. J. Dowson of Tasmania. To both of these gentlemen my cordial thanks are due. The material includes a part of the main stem of a small, stunted plant about nineteen years old (assuming the growth rings to be

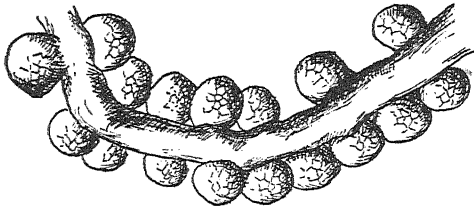


FIG. 1.

FIG. 1. Rootlet of *Pherosphaera Hookeriana*, showing nodules. $\times 24$.

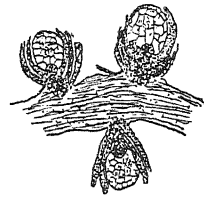
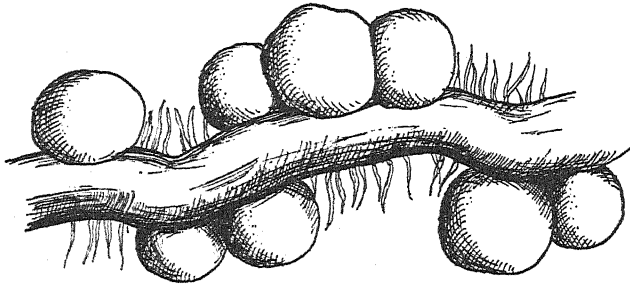


FIG. 2.

FIG. 2. Median longitudinal section of part of a similar nodule. $\times 30$.

FIG. 3. Rootlet of *Podocarpus alpina*. $\times 24$.

annual) and numerous leafy twigs, as well as a quantity of root material. The finer roots, which are very numerous, are almost uniformly covered by a double row of small root nodules (Figs. 1 and 2) quite similar to those described and figured for other Podocarps by Spratt (10) and particularly like those of *Microcachrys*. They average about 0.275 mm. in diameter.

The earlier investigators, such as Nobbe and Hiltner (6) and Shibata (9), believed that the nodules were formed by the activity of a mycorrhizal fungus, the hyphae of which subsequently broke down in the central thin-walled tissue of the nodule. Spratt, on the other hand, working with cultivated material, considered the threads to be zooglea threads of *Pseudomonas radicola*, and she only exceptionally found fungal hyphae, and then only in the outer zone. She also isolated *Pseudomonas* from sterilized material. Subsequent writers (Sahni (8), McLuckie (5)) have confirmed her results.

In the present material of *Pherosphaera* the infective material of the nodule is usually so disorganized that no clear indication remains of its original nature, and this is found to be invariably the case in the nodules of

Podocarpus alpina (Fig. 3), received from the same source and examined for comparison, but no bacteria could be found in either case, and the broken-down threads looked much like the degenerating mycorrhiza of, for instance,

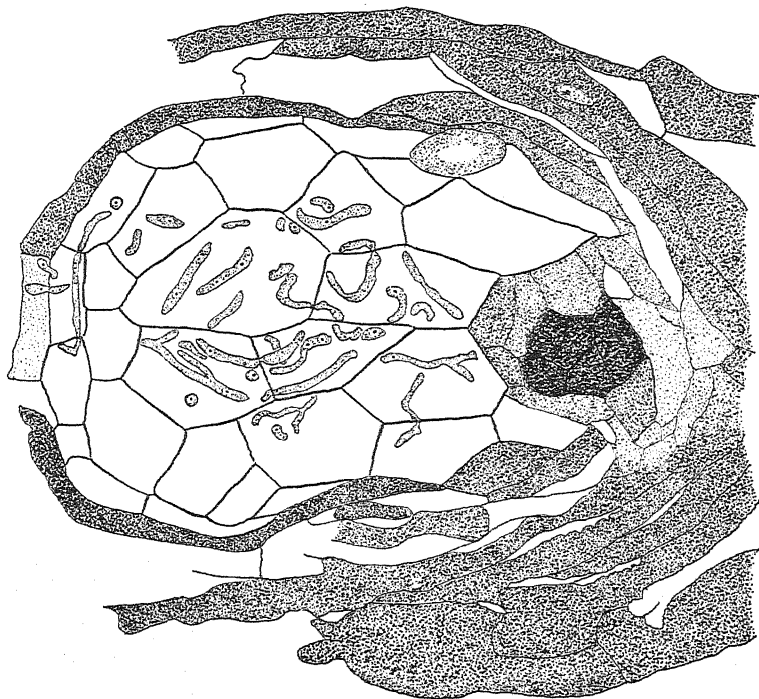


FIG. 4. A nodule from Fig. 2. $\times 350$.

Neottia. Here and there, however, a nodule of *Pherosphaera* was found in which absolutely clear and unmistakable fungal hyphae were present (Fig. 4), and in which there were no threads which could possibly be regarded as bacterial. As all stages between these hyphae and the disorganized remains seen in older stages were found, it is concluded that the nodules can be formed from mycorrhizal infection alone. The later stages appear identical with those of *Podocarpus alpina*, so that the infective material there also would appear to be fungal in origin. In view of the very strong evidence found by Spratt for the presence of nitrogen-fixing bacteria it seems possible that such bacteria follow the mycorrhizal infection, in cultivation, in places where they are plentiful in the soil, but may be absent in the natural habitat of *Pherosphaera*, and very probably of *Podocarpus alpina*. I am much indebted to Professor R. S. Adamson for detailed information in regard to the habitat of these plants, which appears to lend considerable support to the conclusion already reached from a study of the material. Some information regarding the habitat of these plants is also given by Gibbs (1).

Pherosphaera occurs at an altitude of from 3,000 to 4,000 feet, and is confined to a zone about ten yards wide on the margin of peaty areas in the alpine moors of Tasmania. Although rainfall is heavy the habitat is likely to be physiologically xerophytic. The soil is probably of the type

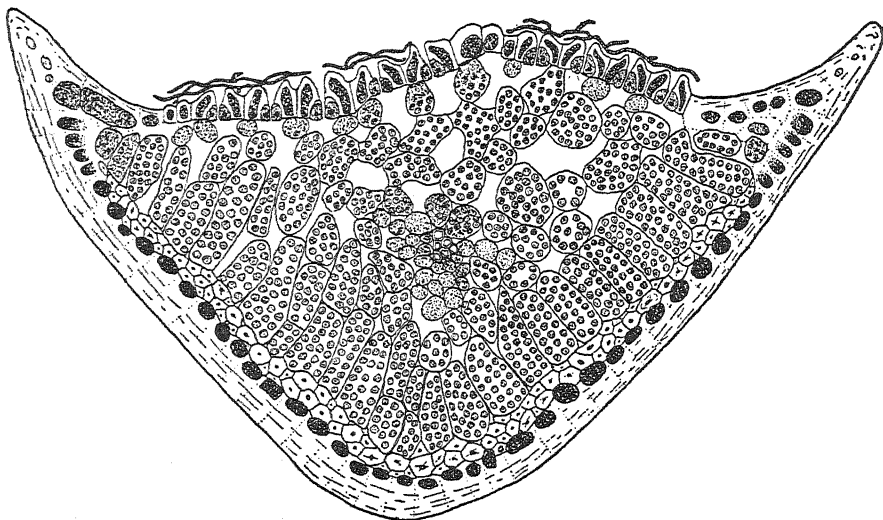


FIG. 5. Transverse section of a leaf of *Pherosphaera Hookeriana*. $\times 200$.

studied by Hesselman (3) in Sweden in 1917, where he found typical nitrification to be absent, ammonia only being formed from nitrogenous organic substances. No Leguminosae grow in association with *Pherosphaera*, and it is by no means improbable that *Pseudomonas radicola* may be quite wanting in the soil. *Podocarpus alpina* grows on or among rocks, and here again no Leguminosae are associated with it, so that in this case also it is at least possible that *Pseudomonas* may not be present.

Whether or not the above conclusions are justified, in any event it seems hardly possible that the nodules of *Pherosphaera* are of a different nature to those of other Podocarpaceae, and that fact furnishes strong evidence for concluding that the genus should remain within the family Podocarpaceae in spite of the peculiarities noted by Lawson (l.c.) in its life-history.

The leaf and stem were also examined. A large proportion of the flat upper surface of the leaf (Fig. 5) is occupied by stomata, and over the stomatal area is a fine network of slender, much branched, and very dark coloured fungal hyphae. The rest of the leaf is covered by an extremely thick cuticle, and below the epidermis is a fibrous hypoderm about two cells thick. Beneath the hypoderm is the palisade, which extends round the whole of the lower surface. Thus in these small appressed leaves the

usual structure of a normal dorsiventral leaf is reversed, the stomata being above and the palisade below, instead of *vice versa*. The vascular tissue is rather rudimentary, and no recognizable phloem can be seen. In the decurrent part of the leaf a resin cavity is found below the bundle, but it

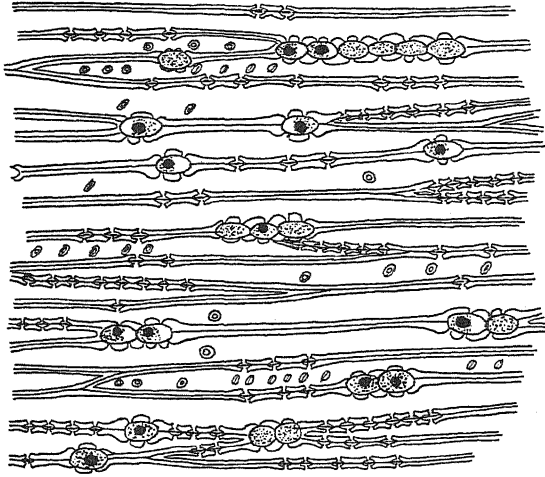


FIG. 6. Tangential section of wood of *P. Hookeriana*. $\times 300$.

hardly extends into the free portion. The margins of the leaf are sharply angular, almost winged, in contrast with the rounded leaf of *P. Fitzgeraldi* as figured by Groom (2), but several anatomical features are common to both species.

The extremely xerophytic character of the leaf is remarkable in view of the rather heavy rainfall, but may be accounted for by the physiologically dry habitat. No other Conifer is known where transpiration is hindered by a network of fungal hyphae over the stomatal area; the hyphae are similar to those penetrating the root nodules, but there is no evidence available which would indicate whether they are identical.

The structure of the stem is very similar to that of *P. Fitzgeraldi* as described by Groom (2). The growth rings are very irregular, some being quite thick on one side and disappearing entirely on another side of the stem. The thicker parts of such rings are composed chiefly of wood fibres. In several cases the spring and summer wood of a ring is represented by only a single layer of cells of each type. The wood cylinder measures 2 mm. in diameter in one plane and 1.5 mm. in the other, the average width of a single growth ring being 0.05 mm. The medullary rays, as seen in tangential section (Fig. 6), are always uniseriate and composed of parenchymatous cells only. The number of cells in vertical series is small, often only one or two cells, though up to six may be found exceptionally.

The same figure indicates the rather large number of bordered pits which occur on the tangential walls. In radial section (Fig. 7) the distribution of bordered pits on the parts of tracheids not in contact with medullary rays is rather variable, and considerable lengths may lack pits entirely.

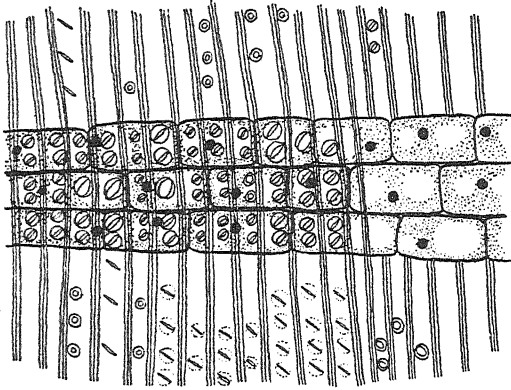


FIG. 7. Radial section of wood of *P. Hookeriana*. $\times 350$.

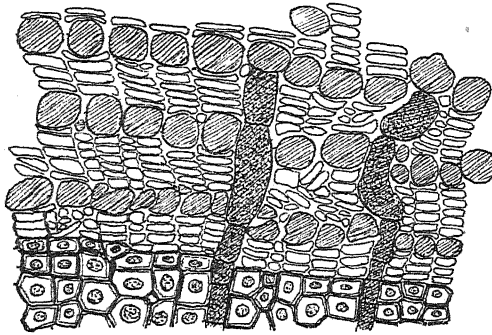


FIG. 8. Phloem, in transverse section of the stem of *P. Hookeriana*. $\times 315$.

In the medullary rays most 'fields' enclose two pits with obliquely fusiform pores, while a few have a single large pit. Thus the leading features of the wood are distinctly Podocarpean. The structure of the phloem is remarkable. It is composed of regularly alternating layers, a single layer of large cylindrical cells alternating with about six or seven layers of very small, flattened, cambium-like elements (Fig. 8). Both types are thin-walled and possess deeply staining contents. In longitudinal section both kinds of cells are seen to be considerably elongated and of about the same length.

It is evident that the anatomical structure and the presence of root nodules strongly support the view that *Pherosphaera* should remain within the Podocarpaceae, but in consideration of the peculiarities in the ovular

development the total absence of prothallial cells in the pollen grain, and the erect axillary ovule with no epimatium, the retention of Pilger's (7) sub-family *Pherosphaeroideae* within the Podocarpaceae, to include *Pherosphaera* alone, seems to be justified.

Thanks are due to Miss L. E. Hawker for the drawings of Figs. 1 and 3, and to Mr. J. B. Cuthbert for cutting the sections from which Fig. 5 was drawn.

Since this paper was completed, root material of *Pherosphaera Fitzgeraldi* has been seen in the Botany Department of the University of Capetown, collected by Professor R. H. Compton at Wentworth Falls, N.S.W., in 1915. This shows unmistakable root tubercles, similar to those of *P. Hookeriana*, but much less numerous. This material has not been examined anatomically.

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Further Studies of the Brown-rot Fungi.

V. Brown-rot Blossom Wilt of Pear Trees.

BY

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With Plates **XLI** and **XLII**.

INTRODUCTION.

PRIOR to the publication, in 1900, of Woronin's (10) paper on the fruit-rotting *Sclerotinias*, it was generally considered that the brown-rot diseases of fruit trees were caused by one species of fungus, *Monilia fructigena*, Pers. = *Sclerotinia fructigena* (Pers.) Schröter. Woronin showed clearly that in Europe two fungi were responsible for these diseases, and that *Sclerotinia cinerea* (Bon.) Schröt. was quite distinct from *S. fructigena* proper, in the morphology of its conidial fructifications and in its relation to the host plants. The ascigerous fructifications of these fungi had not, at that time, been recorded.

According to Woronin *S. cinerea* was confined almost exclusively to the stone fruits, whilst *S. fructigena* infected chiefly the core fruits. More recent observations have shown, however, that the host relations of these species are not so restricted as was formerly thought. Thus in 1917 a serious blossom wilt of apple trees in Britain, caused by a form of *S. cinerea*, was described (6). Moreover in recent years *S. fructigena* has been responsible for considerable rotting of the stone fruits, and in some instances the fruit rot of plums caused by this fungus has far exceeded that produced by *S. cinerea*.

It is true that on apples and pears brown rot of the fruit is caused almost solely by *S. fructigena*; on these hosts *S. cinerea* is rarely associated with fruit rot. *S. fructigena* is not known to cause natural infection of flowers and is therefore not associated with blossom wilts. *S. cinerea*, on the other hand, not only infects the fruit of *Prunus* spp., but also infects flowers and causes blossom wilt of plum, cherry (particularly the Morello cherry), apple, and, as shown below, of pear trees.

That *S. cinerea* is able to infect pear trees appears to have been observed first by Jackson, who, in 1915, published a short description of a pear canker in Oregon (5). The fungus associated with this disease was given the name *Monilia oregonensis*, Barss and Posey, but it has since been shown that this fungus is indistinguishable from *Sclerotinia cinerea* (Bon.) Schröter (9).

The only description that I have been able to find of a similar disease on pear trees in Europe, is a brief note which appeared in 1928 mentioning *Monilia fructigena* as infecting flowers of apple and pear in Italy (1); the colour of the fungus, however, is given as grey ('grigio'), so that probably the fungus was *M. cinerea*. It should be mentioned, too, that Eriksson (4), in describing a brown-rot blossom wilt of apples in Sweden, states that a grower informed him of a similar disease on pears, but no details are given.

SCLEROTINIA CINEREA ON PEAR TREES IN ENGLAND.

The association of *S. cinerea* with diseases on pear trees in England first came under my own observations in 1915 on finding, on a pear tree in a plantation in East Kent, a young fruit, about half an inch long, bearing small grey pustules typical of the *Monilia* stage of *S. cinerea*.

A similar small pear fruit bearing fructifications of *Monilia cinerea* was sent to me in 1921 by Mr. A. D. Cotton, who had received the specimen from Exeter. The dimensions of the conidia on this pear showed variation from 11.5×8 to $28 \times 20 \mu$ with an average of $18 \times 12.5 \mu$. These dimensions are of the same order as those of the conidia of *S. cinerea* as found on infected plums in summer.

A blossom wilt of pears associated with the presence of *S. cinerea* on the flowers was brought to my notice in 1920, also by Mr. Cotton,¹ who sent me a truss of withered flowers and leaves from Cambridgeshire; the variety of pear was not ascertained. Small grey *Monilia* pustules were present on the flowers (calyx tube and pedicels) and on the axis of the inflorescence. The conidia measured 9×6.5 to $19.5 \times 13 \mu$, average $16.5 \times 9.5 \mu$. The mode of germination of these conidia on prune agar plates, and the form of the mycelial growth were similar to those of *S. cinerea* found on plums.

In June 1921 young pear trees of the variety Fertility, growing in the plantation of the South-Eastern Agricultural College, Wye, were seen to be infested with blossom wilt. One tree bore 22 wilted inflorescences, or about 4 per cent. of the total number. All the flowers and leaves on each infected spur were withered, but the infection did not extend further than the base of the spur itself, so that there were no cankers on the branches.

¹ The author takes this opportunity of acknowledging Mr. Cotton's help in providing specimens.

Monilia pustules were present on some of the flowers. Conidia taken from one of these pustules measured 9×8 to 24×12 and $17.5 \times 15 \mu$ with an average of $16 \times 11 \mu$.

On attempting to obtain cultures by isolating conidia on culture plates, there was found to be so much contamination from bacteria that that method was abandoned and plates were prepared by sterilizing the outer surface of an infected spur and then transferring particles of the internal tissues to prune agar plates. From these particles hyphae grew out and produced cultures which were, apparently, quite pure. Such cultures on prune agar did not produce spores, but inoculations from them on to sterilized potato resulted in a good crop of conidia within seven days, and from these conidia monospore cultures were obtained.

During the winter of 1921-2 the trees were subjected to the usual routine pruning, but when they were examined in February 1922 a number of the spurs, which had been overlooked during the pruning operations, remained, and these bore grey *Monilia* pustules. The conidia on these pustules were much smaller than those found on the flowers the previous season. Their dimensions were 8.5×6 to 14×10 and $15 \times 9.5 \mu$, with an average of $11.5 \times 8 \mu$. These dimensions, again, are in conformity with those recorded for *S. cinerea* f. *pruni* on plums and for *S. cinerea* f. *mali* on apple spurs, when produced on pustules which develop during the winter (8). Monospore cultures were obtained by isolating conidia taken from these winter pustules on a dead pear spur, and the resulting cultures were similar to those obtained from the tissues of a spur in the previous June. In May, 1922, a few infected inflorescences were found on two of the trees, the infection, it must be assumed, being caused by conidia produced on those spurs which had been killed the previous year and not removed from the trees.

In 1925 Mr. J. Amos collected specimens of pear blossom wilt from Fertility pear trees at Wisborough Green, Sussex. He estimated that there were some seventy or eighty infected trusses per tree on trees six to seven feet high. One of these specimens showed a dead terminal spur (result of infection in 1924) bearing pustules, and a few inches below it were two wilted inflorescences recently infected, also bearing pustules (see Pl. XLII, Fig. 4). The fungal fructifications of the old spur were on the flowers, pedicels, petioles, and the axis of the spur; on the current year's inflorescences the pustules were on the calyx tubes and pedicels only.

Field observations have shown, then, that Fertility pear trees are subject to a blossom wilt associated with the appearance of the *Monilia* stage of *S. cinerea* on the dead flowers and spurs. As has been noticed in other cases of infection by this species the conidia produced on the spurs in the winter and early spring are noticeably smaller than those which develop on the newly infected flowers during the summer. In culture the

fungus resembles *S. cinerea* forma *pruni* rather than forma *mali* in that the dark coloration, characteristic of the latter in prune agar cultures, was lacking, and also that on sterilized potato there was copious development of conidia, not scanty as in the apple blossom wilt fungus.

The constant association of *S. cinerea* with this form of blossom wilt in pears, and the fact that forms of this fungus are known parasites on other kinds of fruit trees were good presumptive evidence that the fungus was the cause of the wilt, but experimental proof was required for confirmation, particularly as a bacterial disease of pears, described by Barker and Grove (2), produces somewhat similar symptoms. Again, since the fungus is morphologically and culturally similar to *S. cinerea* f. *pruni* it was necessary to determine whether *S. cinerea* as found on plums and cherries is able to infect pear flowers.

INOCULATION EXPERIMENTS.

(A) *Inoculation of Pear Flowers.*

Attempts to prove experimentally that the fungus is the cause of the blossom wilt were at first unsuccessful. All inoculations of flowers on Fertility pear trees in the open, using conidia of strains from naturally infected pear inflorescences, have given negative results. Such inoculations have been carried out in the field during four different seasons, but in no case did blossom wilt occur.

In 1924 greenhouse accommodation and suitable trees were available at the East Malling Research Station, so inoculations were carried out under glass. A fairly moist atmosphere was maintained by watering the floor of the greenhouse frequently, and the trees were occasionally sprayed with water. The inoculations were made with conidia taken from a pure culture of *S. cinerea* isolated from an infected pear spur, and grown on sterilized potato.

In order to ascertain what parts of the flower were most susceptible to infection, conidia were placed on (1) the disc, (2) petals, (3) receptacle, and (4) stigmas. The conidia were transferred from the culture to the organs to be inoculated by camel hair pencils which had previously been boiled for five minutes and allowed to cool. The pencils were used slightly moist so as to avoid the possible dispersal of the spores by currents of air. The results were as follows:

(1) *Inoculation of the floral disc.* Seventeen flowers inoculated. There was no definite evidence of infection, though in some flowers a slight discoloration of the disc was observed which was not to be seen in flowers not inoculated.

(2) *Inoculation of petals.* Two to five flowers on each of eight inflorescences were inoculated on the lowest two petals; the flowers were

first sprayed with sterilized distilled water, and conidia were then placed in the drops of water which collected on the concave inner surface of the lower petals. In all, 29 flowers (58 petals) were inoculated. Many of the inoculated petals showed a browning at the inoculated spots in two or three days, the spots in some cases being several centimetres in diameter, but, with one exception, all the inoculated petals fell off before the infection had extended into the base of the petals.

The one flower which eventually became infected was inoculated on two petals on April 30, the day the flower opened. Within two days each petal showed a brown blotch at the place of inoculation, and this had increased in size by the following day, when, however, one of the petals fell. The other inoculated petal remained attached, and on May 4 the browning had extended to the receptacle and to the two sepals nearest to the infected petals; the distal half of the petal was still white and unaffected. On May 5 the discoloration had extended into the pedicel, and by the next day had reached the base of the pedicel; the infected petal remained attached to the flower.

May 8. The whole axis of the inflorescence was discoloured; the leaves and the rest of the flowers were becoming blackened from the base upwards for 5 to 8 mm. and were flagging.

May 9. The whole truss was withered; conidial fructifications were present on some of the pedicels.

Although there was only this single example of definite infection there is good evidence that it was the result of inoculation, as shown by, (a) the discoloration of many of the inoculated petals before they fell, such browning not appearing on petals not inoculated, even on the same flower; (b) the progressive infection of the organs from the inoculated petal to the axis of the inflorescence; and (c) the appearance finally of *Monilia* fructifications on the flowers of the infected inflorescence.

(3) *Inoculation of the receptacle.* One to three flowers on each of four inflorescences were inoculated by making a puncture on the side of the receptacle and touching the spot with a brush bearing conidia. The inoculations were made on April 26, and control punctures were also made.

Although a slight discoloration was observed round some of the inoculated punctures there was no evidence of definite infection after ten days.

(4) *Inoculations of stigmas.* Six inflorescences were selected, and two, three, or four flowers on each inflorescence were inoculated on the stigmas, 21 flowers in all.

All the inoculated flowers showed the early stages of infection. There was first a browning of the stigmas; this gradually became more noticeable and then the discoloration passed down the styles until these were completely brown. The discoloration of the floral disc followed.

On one inflorescence infection ceased at this stage. On the others, however, infection soon appeared in the receptacle, and passed along the pedicel into the axis of the inflorescence. This was followed by the wilting of all the flowers and leaves on the spur, the result being typical blossom wilt.

The rate of progress of the infection (as shown by the discoloration) was fairly uniform throughout, and in general the symptoms noted, in relation to the length of time after inoculation, were as follows :

No. of days after inoculation.	Result.
3	Stigmas brown.
6	Styles (one or more) brown to base.
7	Disc discoloured.
9	Receptacle and part of pedicel infected.
10	Pedicels discoloured to base.
11	Axis of inflorescence invaded : leaves and flowers flagging.
12	Leaves and flowers completely wilted.

It will be seen that the rate of progress was very slow during the first week. About the seventh day the infection had reached the disc at the base of the styles. After this progress was more rapid, probably as a result of the stimulus afforded by the foodstuffs in the nectar and in the tissues of the ovary. The pedicels of the flowers were 1.5 to 2 cm. long, and when the discoloration appeared at the distal end of a pedicel it extended towards the base at the rate of about 1 cm. per day, so that the axis of the inflorescence was then soon invaded.

In the infected flowers the calyx lobes and stamens remained turgid and healthy looking until the infection had reached the axis of the inflorescence, when the whole flower withered.

At the time that an inflorescence as a whole began to flag, the uninoculated flowers on it showed no discoloration except at the base, where they were being invaded from the axis; the petals by this time had fallen, but the styles, stamens, and sepals were turgid and quite normal in appearance.

Owing to the absence of pollination under the conditions of the experiment the styles of flowers not inoculated generally showed no discoloration at this stage, though a slight browning of the stigmas of some flowers was to be observed.

Later the tree was placed in the open. During the winter the infected spurs could be distinguished from the rest by the persisting withered flowers and leaf-stalks. Pustules of the *Monilia* stage of *S. cinerea* appeared on the withered organs during winter and spring (see Pl. XLII, Fig. 7).

In 1925 inoculations were again made on pear flowers in the greenhouse, using a pear strain of the fungus. The early symptoms of infection

were seen in the browning of the styles in most cases, but the flowers fell without any infection of the spurs. Probably in this experiment the air in the greenhouse was too dry for the infection to run its course.

In 1926 another series of experiments was carried out on pear flowers using a cherry strain, a plum strain, and, for comparison the pear strain used in previous experiments. Again conditions for infection were not wholly favourable, for although flowers on nine inflorescences were inoculated with each strain, and most of them showed definite evidence of infection, the discoloration extending into the receptacle and also, on some flowers, into the pedicel, only one spur was killed. The flowers of this inflorescence in which the infection was complete had been inoculated with the cherry strain.

A similar series in 1927 gave even less satisfactory results, most of the inoculated flowers showing merely infection of the styles; only two flowers (inoculated with a plum strain) showed infection of the disc and receptacle.

In 1928 a greenhouse more suitable for pathological experiments was available, and the inoculations were continued. Successful infections were obtained with a pear strain and also with a strain of *S. cinerea* isolated from a plum twig. With the latter five inflorescences were inoculated on two flowers each, and all the ten flowers became infected. The spurs were invaded and typical blossom wilt resulted. In a similar series of inoculations on five inflorescences, using a strain from a plum fruit, all the ten inoculated flowers became infected (producing at least a blackening of the disc), but only one spur was killed.

The trees on which these experiments had been carried out were placed in the open, where they remained over the winter. When they were examined in April, 1929, it was seen that some of the infected spurs bore *Monilia* fructifications which had developed during the winter and spring. Conidia taken from one of the spurs which had been inoculated with the pear strain were found to measure 8.5×6 to 12.5×10.5 and $14 \times 8.5 \mu$, with an average (100 conidia) of $10.5 \times 7.5 \mu$. It will be seen that these dimensions correspond to those of conidia obtained from naturally infected spurs during the winter months.

Convincing proof that a strain of *S. cinerea* isolated from a cherry can infect pear flowers was obtained in 1929. The inoculations were made on a young Fertility pear tree in the greenhouse. Ten inflorescences were inoculated on two flowers each. All the flowers became infected, in every case the discoloration extending to the base of the pedicel. On three inflorescences, however, the flower-bearing axis was not invaded. On one other the flower-bearing axis was blackened and killed, but the infection did not extend into the older part of the spur bearing leaves, and the latter remained alive and healthy. In the remaining six the spurs were killed to their base so that the flowers and leaves all wilted, the symptoms being characteristic of typical blossom wilt.

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EXPLANATION OF PLATES XLI AND XLII.

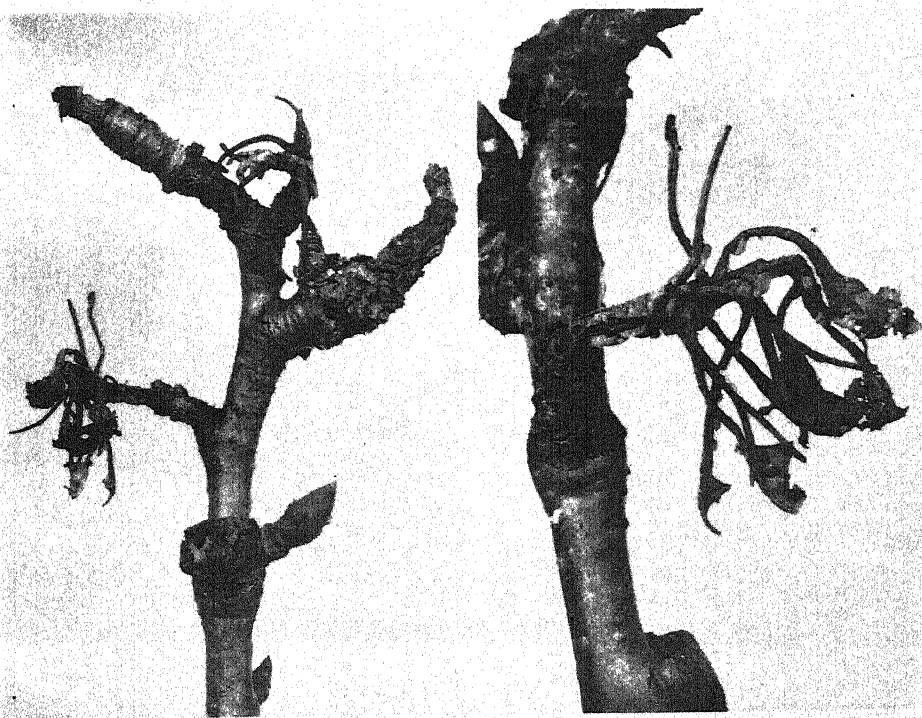
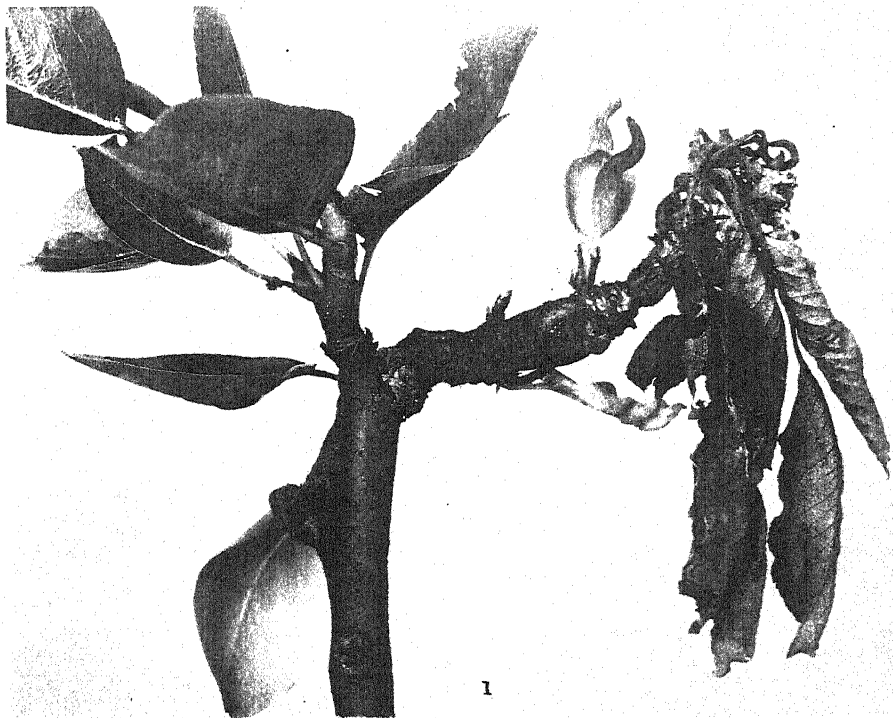
Illustrating Dr. H. Wormald's paper on Further Studies of the Brown-rot Fungi.
V. Brown-rot Blossom Wilt of Pear Trees.

PLATE XLI.

- Fig. 1. Pear blossom wilt, summer condition (natural infection).
 Fig. 2. Pear blossom wilt, winter condition, showing two infected spurs (natural infection).
 Fig. 3. One of the spurs shown in Fig. 2, enlarged ($\times 2$) to show conidial fructifications of *Sclerotinia cinerea* on the withered flowers and leaves.

PLATE XLII.

- Fig. 4. Natural infections on Fertility pear. Above is a dead spur infected the previous year, and below are two inflorescences recently infected.
 Fig. 5. The branch on the left bears four infected inflorescences, the result of inoculation with conidia from a pure culture of a strain of *S. cinerea* isolated from a pear spur.
 Fig. 6. A pear inflorescence showing an infected flower in the centre; this flower had been artificially inoculated with conidia of *S. cinerea*; note the blackened pedicel and the withered stamens and calyx lobes.
 Fig. 7. A dead pear spur in March, 1925. Flowers of this spur had been artificially inoculated with *S. cinerea* in April, 1924.
 Fig. 8. A pear tree bearing a number of withered inflorescences, each of which had been inoculated on two flowers with conidia of a cherry strain of *S. cinerea*.



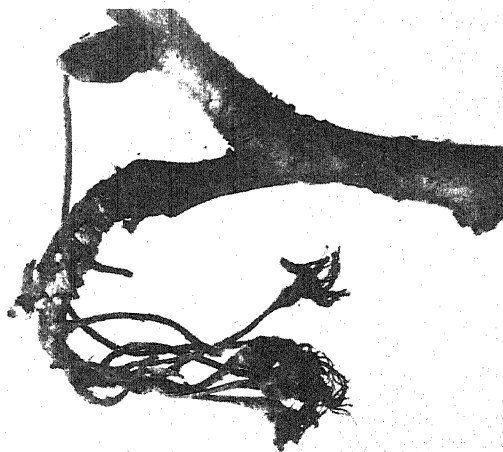


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6



7

WORMALD — BROWN ROT BLOSSOM WILT.

Huth coll.

Saltations in Bacteria.¹

III. *Bacillus violaceus*.

BY

J. C. RAMCHANDANI.

(From the Bacteriological Laboratory, Imperial College of Science and Technology, London.)

With Plate XLIII.

THE investigations of which the results are to be given in this paper form a continuation of the work done in this laboratory by Nirula (22) and extended on a strain of *Bacillus prodigiosus* by the author (26). Nirula found that the variants which he obtained remained constant and that reversion could not be induced by any methods of cultivation, and that these variants were sufficiently differentiated from the parent as to suggest that in the course of evolution new species may have arisen through similar processes of saltation. The author obtained from *B. prodigiosus* two white strains and found that they also remained constant and resembled the parent morphologically, physiologically, and serologically. The most striking feature that arose in this work was the suggestion of loss of pathogenicity for the mouse exhibited by one of these white strains. This relationship of microbic dissociation to virulence has been noticed by various workers, but De Kruif (7) was the first to demonstrate it definitely. It has, however, commonly been found that it is the saltant or 'R' type which is non-virulent.

The above work on *B. prodigiosus* having given such promising results, the author decided to continue similar experiments on some other highly pigmented bacterium. *B. violaceus* was selected for the following two reasons: firstly, it developed a very dark violet pigment, thus giving a good background for the differentiated cells to show their colour characteristics; secondly, it might bring to light some phenomena which had not arisen in the previous research on *B. prodigiosus*.

¹ Saltations in Bacteria, by R. L. Nirula, which appeared in this Journal, vol. xlii, pp. 431-66, 1928, forms Part I of this series.

Saltations in Bacteria, by J. C. Ramchandani, which appeared in this Journal, vol. xliii, pp. 579-86, 1929, forms Part II of this series.

EXPERIMENTAL.

The strain of *B. violaceus* employed in the following investigation originally came from Nigeria and was obtained from the Collection of Type Cultures at the Lister Institute, where it had been maintained for some time as stock culture No. 2537. As in the previous work, single cell cultures were obtained by the method described by Paine and the author (23). It had been noticed when working on *B. prodigiosus* that certain factors favoured saltation, namely suitability of temperature, food substances, and volume of medium. It had been found, for instance, that optimum temperature and Brown's (6) synthetic agar favoured maximum sectoring. There is no doubt, however, that different species of bacteria are affected differently by the same factors. A medium which might serve usefully for *B. prodigiosus* might not produce much effect upon *B. violaceus*, therefore in order to increase the chances of success in obtaining saltation with these organisms three different media were employed, numerical preference, however, being given to Brown's medium for the reason just stated. Eighty plates were used in the first experiment, forty-five of these being of Brown's medium, twenty of potato-extract, and the rest of bouillon agar. Transfers from single cell cultures were made in the usual way by point inoculations spaced in each quadrant of the petri dish in order to give them sufficient room to grow uninfluenced by the inhibitory substances produced by the neighbouring colonies. As previous records of the characteristics of *B. violaceus* place its optimum temperature for growth somewhere between 25–30°C., all the plates were incubated at 25°C. On the third day it was observed that there was a remarkable difference in the colour which developed on the three media employed, it being dark violet on bouillon agar, pale purple on potato-extract agar, and pale pink on Brown's synthetic agar. It was thought possible that this reduction in the intensity of the colour on the latter two media might not have occurred had the incubation of the cultures taken place in the light, so half of the total number of plates of each medium were wrapped in black paper and the rest left uncovered near a window. After four days' incubation in this way no difference could be observed between the two sets except a marked zonation in the case of the uncovered set as compared with the other. It was concluded from this that as regards intensity of colour daylight was without influence, and all the plates were put back into the incubator to allow of the formation of giant colonies. The first appearance of sectors was noticed on potato-extract medium and was observed eight days after the setting up of the experiment; four colonies showed the presence of seven sectors. These colonies were kept under close observation in order to see if, in comparison with other colonies, they

had any special tendency to produce sectors. Seven more sectors appeared in these colonies while the majority of the other colonies, of which there were a total of eighty, remained without saltation, even on the seventeenth day. Only three more colonies had produced sectors. In one of the plates the four colonies differed rather markedly from one another; three of the colonies had an entire margin while the fourth had a serrated margin. It was observed that the serration of the margin was due to the presence of a large number of tiny sectors. Two of the colonies with entire margin showed no sign of dissociation, while the remaining one had a white margin formed by sectors which had coalesced to form a complete ring. This multiple development of sectors in certain colonies compared with the complete absence in the others had been noticed in the case of *B. prodigiosus*, and it was believed that it might be due to a few differentiated cells possessing a tendency to saltate, but the question had not been looked into at that time. At the setting up of the first experiment in the present research special attention was focused upon this point. The following table confirms the suspicions entertained by the author at the time of the previous research. This experiment was repeated and confirmed.

TABLE I.

Sectoring tendencies of different colonies on potato-extract medium. Number of plates twenty, each carrying four colonies, one in each quadrant. Thickness of medium in each plate approximately 6.0 mm. Temperature of incubation 25° C.

Plate No.	No. of sectors on the 8th day.	No. of sectors on the 10th day.	No. of sectors on the 12th day.	No. of sectors on the 17th day.
Pl. I.				
Colony 1.	2	3	3	3
" 2.	0	0	0	0
" 3.	0	0	0	0
" 4.	0	0	0	0
Pl. II.				
Colony 1.	1	2	3	0
" 2.	0	0	0	0
" 3.	0	0	0	0
" 4.	0	0	0	0
Pl. III.				
Colony 1.	3	4	5	5
" 2.	0	0	0	0
" 3.	0	0	0	0
" 4.	0	0	0	0
Pl. IV.				
Colony 1.	1	3	3	3
" 2.	0	0	0	0
" 3.	0	0	0	0
" 4.	0	0	0	0

TABLE I (continued).

Plate No.	No. of sectors on the 8th day.	No. of sectors on the 10th day.	No. of sectors on the 12th day.	No. of sectors on the 17th day.
Pl. V.				
Colony 1.	0	0	0	2
" 2.	0	0	0	1
" 3.	0	0	0	0
" 4.	0	0	0	0
Pl. VI.				
Colony 1.	0	0	0	2
" 2.	0	0	0	0
" 3.	0	0	0	0
" 4.	0	0	0	0

The colonies on the remaining plates in this set did not throw any sectors at all, even after every encouragement to do so had been given them by prolonged incubation. In the case of bouillon agar, two colonies only threw sectors, and even these gave but one sector each; while on Brown's medium, sectors were not noticed till late, as on this medium the parent hardly develops any pigment, although most of the colonies showed a sectoring tendency.

In this experiment all the sectors looked very much alike, and the author was led to believe that there was only one saltant. To separate the strain and to make comparison of its properties with those of the parent, subcultures were made in the same plate both from the parent and the sector part of the same colony in the following media: bouillon agar (A), Brown's synthetic agar (B), potato-extract agar (P.E.), glucose-peptone agar (G.P.), potato-extract-glucose agar (P.E.G.), potato-extract-peptone agar (P.E.P.), and potato-extract-glucose-peptone agar (P.E.G.P.). Seventy plates were used, ten of each medium, and were incubated at 24°, 30°, and 37° C. As mentioned above, the amount of pigment produced varied in different media, being dark violet in media A and P.E.P., violet in G.P. and P.E.G.P., and pale purple in B, P.E., and P.E.G. It was noticed that the potatoes used in the second experiment did not favour the development of pigment as much as those used in the first experiment, although the method of preparing the medium had been the same. The colonies which arose from the sector part were also affected differently by different media. They remained white in media B, P.E., and P.E.G., giving the impression that the saltant bred true, while they developed dark purple pigment in media A and P.E.P., and purple in G.P. and P.E.G.P. The colonies were not uniform in colour but were streaked with the colour of the violet parent strain (Pl. XLIII, Fig. 1).

At this stage, sectors of three different shades, dark violet, purple, and white, were observed in the parent colonies, although they could not be

differentiated in every medium. On subculturing only the white one bred true.

It is quite possible, in fact probable, that the dark violet may be a saltant, but its pigment differed so slightly from that of the parent strain that on subculturing from it the colour of the colonies was indistinguishable from that of the colonies of the parent, and further it was not found possible to differentiate the colonies by any other phenomenon. The colour was supposed at this time to be a mixture of white and violet, and it was so uniform that the impression was gained that to produce it there existed a certain ratio between the parent and the white strain. Had this been the case it should have been possible to initiate the colour of the saltant by inducing a joint development of the parent and the white saltant in the same ratio. Attempts were made to reproduce the purple colour in colonies from mixed suspensions of the violet and white strains. Suspensions of equal density of the violet parent, the white saltant, and the purple sector were made in sterile broth and incubated at 20° C., to get a uniform suspension. A series of nine tubes each containing 10 c.c. of sterile broth were mixed with the following proportions of the parent and the white strain: nine loops of violet to one of white, 8-2, 7-3, 6-4, 5-5, 4-6, 3-7, 2-8, and 1-9. Inoculations from the parent, from the white and purple strains, and from each of the nine mixtures were made in the same bouillon agar plates. Three days after the commencement of the experiment no difference whatsoever could be observed between the purple and the white strains, but after the third day the purple one developed its pigment. None of the ratios employed showed any resemblance to the purple colony (Pl. XLIII, Fig. 2). At a later stage, single cell cultures were obtained of both white and purple strains, and when plates were poured from a pure purple colony all the colonies developed a uniform purple colour, while the plates poured from the mixed culture of violet and white always developed colonies of the two representative groups. This led the author to assume that it was a different strain from the white one. In order to determine whether these strains would saltate further, transfers from the parent and the purple saltant were made in one set of plates and the parent and the white saltant in another, using the media A, P.E., P.E.P., P.E.G., and P.E.G.P. Fifteen plates of each medium were used and incubated at 24°, 30°, and 37° C. After fifteen days, there appeared a new type of white sector of a matt surface on one of the white colonies growing on medium P.E.G. at 30° C. Later, two more sectors of the same type appeared on the same colony. After twenty-four days this colony gave also a more transparent white sector (Pl. XLIII, Fig. 3). One had then the original strain of *B. violaceus*, *V*; the first saltant purple strain, *V*₁; the second saltant white strain, *V*₂; the third saltant white strain, *V*_{2a}; and the fourth saltant white strain, *V*_{2b}. These five strains were compared most carefully by culture on various media and

different temperatures to determine if any differences existed in their morphological, cultural, and physiological characteristics. Transfers of all five strains were made by point inoculations from the respective suspensions of equal density in the same dish. Ten inoculations, three-quarters of an inch apart, were made on four different media, P.E., P.E.G., P.E.P., and P.E.G.P., in order to study the rate of growth and cultural characters.

Sixty plates were used and incubated at 20°, 30°, and 37° C. Measurements and cultural characters were recorded every third day. After nine days, the colonies were so large as to affect each other's growth, so the taking of further measurements could have no significance. Here attention may be drawn to the constant differences in the rate of growth of the various strains. Strains V , V_1 , and V_{2b} were comparatively slower than V_2 and V_{2a} , while V_{2a} was faster than V_2 . It was also observed that the growth rate of V_{2a} was peculiarly affected by the medium P.E.G. as compared with P.E., P.E.P., and P.E.G.P. It developed more slowly in this medium than in the other three for the first three days. At the end of six days, it had grown faster in this medium at 30° and 37° C. than in all the other media except P.E.G.P. It appeared that the strain V_{2a} developed best in the latter medium at 37° C. For all the other strains, medium P.E.P. seemed to be most favourable.

Variation in the rate of growth suggested the possibility of physiological differences among the five strains which might be made manifest in other ways. Various attempts to find such differences showed in the main a remarkably close similarity, the change of H-ion concentration in liquid media for instance which might be expected to reflect any diversity, showed after 40 and 60 hours incubation practically no difference between the five strains. Gelatine stabs, on the other hand, showed rather a remarkable difference in the amount of liquefaction; greatest liquefaction was shown by the parent, while the purple strain liquefied gelatine more than the three white strains (Pl. XLIII, Fig. 4). Another slight difference between them was found in their action on dextrin, V_2 forming acid while the others did not affect it, and further, in most of the sugars, the parent unlike the strains formed a pellicle, a ring, and a thick precipitate. Apart from the above-mentioned colony characters of colour, transparency, rate of growth, gelatine liquefaction, and action on dextrin, no other differences could be observed. There did, however, appear certain points of divergence from the published description of the behaviour of *B. violaceus*. Lehmann and Neumann (19), Bergey (5), and Mehta (20). According to Bergey and Mehta it does not form indol, Mehta also found no acid in glucose and a reduction of nitrate to nitrite. Further, as regards its optimum temperature for growth, in Bergey one finds this given between 25–30° C., while Lehmann and Neumann say that it grows best at ordinary temperature. This difference can be explained by the fact that this strain of *B. violaceus*

originally came from Nigeria and may on that account have higher optimum temperature. Contrary to the author's experience Lehmann and Neumann found its pigment insoluble in ether.¹ It produced acid, but no gas, in media containing glucose and levulose, but no acid in media containing lactose, sucrose, galactose, raffinose, maltose, mannite, dulcitol, sorbitol, inulin, dextrin, and salicin.

By the courtesy of the staff of the Lister Institute in charge of the National Collection of Type Cultures, it has been possible to investigate the serological reactions of these five strains. Immune sera were obtained from four strains, the titre of the parent being 1/1600, that of strain V_1 being 1/3200, that of strain V_{2a} being 1/10000, and that of strain V_{2b} being 1/6400. With each of these sera the five strains reacted in precisely the same manner. It was, however, noticed that the rate of agglutination was different in the different strains, V_{2a} agglutinating quicker than the rest of the strains. Mention is made of this observation, since, possessing very little information as to the physico-chemical nature of agglutination phenomena, the writer is not in a position to judge as to how far such observation may be of importance.

ATTEMPTS TO INDUCE REVERSION IN THE NEW WHITE AND PURPLE STRAINS.

Various methods of culture have been employed with the object of producing, if possible, a return to the pigmented condition in the new strains which have arisen by saltation. In the first series, the effect of temperature was investigated. Nine different media were inoculated, each for two colonies of the parent, two colonies of strain V_1 , two colonies of strain V_2 , two colonies of strain V_{2a} , and two colonies of strain V_{2b} . The special media used were bouillon agar, Brown's synthetic agar, Brown's synthetic agar plus starch and peptone, potato-extract agar, glucose-peptone agar, potato-extract-glucose agar, potato-extract-peptone agar, potato-extract-glucose-peptone agar, and Thornton's medium. Five plates of each were incubated at 20°, 30°, and 37° C. Observations were made at intervals of three days and continued for nine days. There was no reversion of the purple and the white strains to the parent type, but there was partial suppression of colour in the parent strain in Brown's medium, potato-extract agar, potato-extract-glucose agar, and Thornton's medium, and there was complete suppression of colour in the purple strain in the above four media. This suppression of colour appeared to be due to the absence of peptone in those media. The plates were left standing on the table and after four days it was noticed that the colonies representative of V_2 , V_{2a} , and V_{2b} in the medium potato-extract-glucose-peptone agar were showing signs of partial reversion. The margin of V_2 adjacent to that of V_1 had turned purple and

¹ The pigment produced by *Chromobacterium violaceus* has been isolated by Joseph Reilly and Gerald Pyne (27) and shown to be soluble in ether.

V_{2a} had remained white and flat. In the same plate the margin of V_{2b} remote from V_{2a} had turned pale purple and that towards V_{2a} had remained white, and the margin of V_{2a} remote from both V_2 and V_{2b} was turning pale purple. In order to discover if this was a case of reversion, ten plates of medium P.E.G.P. were inoculated from the purple margin and the white margin of V_2 . Fifty more plates of the same medium were inoculated with the pure cultures of V_1 , V_2 , and V_{2b} , either one strain in one plate or two strains in the same plate. All these plates were incubated at 30° C. and examined every day. After nine days, they were found to be quite normal and exhibited their usual characteristics. The colonies from the purple and white margin of the same colony V_2 had grown in the normal V_2 type. At this stage, half the number of plates of each type were taken out from the incubator and left on the table. In every case it was found that V_1 turned dark purple, V_2 had a dirty-white centre surrounded by a pale purple band with a purple margin, V_{2a} had turned very pale purple with a white margin, and V_{2b} resembled V_2 . Further, it was observed that more purple colour developed near the edge of the plate than towards the centre. The plates left in the incubator remained normal. It appeared as if light influenced the production of pigment, but the results of further experiments have cast doubt as to this, and at the time of writing the question remains an unsettled one. The conditions leading to this apparent reversion are being investigated, and the results will be given at some later date.

In the second series the effect of alteration of the concentration of hydrogen-ion was determined. Forty-five plates containing potato-extract-peptone agar in a range of pH 5.0, pH 7.1, and pH 9.0 were incubated at 20° , 30° , and 37° C. No change in the pigmentation of the colonies was observed, but there was the usual difference in the rate of growth between the various strains, and the multiplication of the organisms was greater at the acid end of the range. It may be noted here that sector formation was favoured by neutrality, the greatest number of sectors being found on colonies growing on media of pH 7.1. Further, it was strikingly noticeable that no sectors appeared in the colonies growing on media of pH 5.0. In order to see if the same phenomenon of reversion as shown in the first series occurred in this case, half the number of plates after nine days incubation were left on the table, but no such reversion was observed. Attempts were made to induce reversion by growing purple and white strains very near to the parent. Apart from the restricted growth and flattening of the margin, no effect was observable.

GENERAL OBSERVATIONS.

In the beginning it was observed that the parent strain was non-motile. In order to induce motility transfers were made to broth and back

to bouillon-agar and repeated several times but without the desired result. However, during the course of this investigation the organism has been repeatedly subcultured, and after some thirty such transfers it was observed that the organism had assumed motility. It has been pointed out by Nāgeli (21) as early as 1877 that the motility of bacteria can be lost and regained in this way.

In some of the plates in the above experiments, it was observed that a few colonies of V_1 were showing white and dark purple sectors. Whether these sectors were the same as given by the parent the author had no time to investigate.

It was observed that *B. violaceus* was gradually yielding more and more purple and white sectors (Pl. XLIII, Fig. 5), and the transfers on bouillon-agar slants were becoming paler. It appeared as if the parent was being slowly but surely superseded by its saltant strains. When plates were poured from a culture which had been transferred some thirty times, it was observed that except a few colonies all were either purple or white. Similar phenomenon has been observed by Hadley (14) in the case of *B. pyocyaneus*. He mentions that a pyocyanous culture showing erosive action on a solid culture medium gradually loses its power to produce the blue pyocyanin and, little by little, comes to a stage of a fluoresceinogenic, but non-pyocyanogenic organism. He plated out suspensions in broth at varying intervals and found that, at first, perhaps ninety-nine out of every hundred organisms yielded a rich blue-green colony. But gradually these bacteria were replaced by organisms which produced no pyocyanin whatever. Thus the changing ratio may be 99:1, 75:25, 50:50, 25:75, 1:99 or even 1:100. He says that the change in this biochemical character does not involve 'a little loss' of pyocyanogenic power on the part of all organisms present, but the sudden appearance of a new type of organism. This author does not mention if he ever tried to grow his *B. pyocyaneus* sufficiently long to produce giant colonies. It is quite possible that the same phenomenon of saltations as shown by *B. violaceus* has been going on in his cultures of *B. pyocyaneus*. It may be mentioned here that this phenomenon was observed by the writer also in *B. prodigiosus*.

During the course of this work it has been noticed that cultures of *B. violaceus* died rather quickly, and therefore transfers had to be made every few days. Enderlein (9) has stated that the so-called 'dead' old cultures are not really dead. He refers to the fact that when no growth results when subcultures from old agar slants are made on another slanted medium, it is customary to conclude that the old culture is dead, but, according to him, it is not necessarily so, it might have undergone a transformation. He thinks that at first gonidia are formed which are easily cultivable on fresh media. Later, as the culture ages, the gonidia give rise to gonites which cannot be cultivated on solid media, but which, when

planted in broth, become transformed into the sex cells (spermites and oites). Neither of these forms is cultivable independently, but after fertilization has taken place in a liquid medium, they proceed to generate the normal type. The author attempted to grow a culture of *B. violaceus* which was two months old. Transfers were made in broth and solid medium from the same culture, but no growth was observed in either case.

FACTORS AFFECTING SALTATION.

Temperature. Several previous workers have pointed out that temperatures above the optimum stimulate saltation; Laurent (18) as early as 1890 showed that pigment production in *B. prodigiosus* is influenced by high temperature and this has been confirmed by the author and several others, but the change here has been shown to be an ephemeral one. Permanent dissociations, on the other hand, have been observed in fermentative reactions by Wilson (32) and in virulence by Pasteur, Chamberland and Roux (24), Bail (4), Roux and Yersin (29), and Hewlett and Knight (16). This phenomenon of dissociation due to higher temperatures than normal has been noticed by various other workers. But in the above research it will be seen that saltants arose at the optimum temperature and therefore can not be classed with the above type.

Medium. The influence of the medium in causing mutation has been reported by Penfold (25) when certain organisms were grown on various sugars, also by Stewart (31) when *B. coli mutabile* was grown in the presence of lactose, and further by De Kruif (8) who has shown that high concentrations of peptone favoured dissociation in *B. lepi-septicum*. But the saltants obtained by the author from *B. violaceus* cannot be strictly classed with the above type as they did not appear to be direct adaptations to food substances. It has been pointed out by De Kruif (8), Reimann (28), d'Herelle (15), and Amoss (1) that an acid medium retarded the tendency to dissociate while an alkaline reaction often accelerated it. But, as stated earlier, it was found that for *B. violaceus* a neutral medium was most favourable for saltation while an acid medium prevented the tendency in this direction altogether.

Reversion. Regarding the permanence of saltants, there exists much conflicting evidence. Fritsch (11) was the first who pointed out the permanence of mutants. Various other workers (14) are in agreement with him, while Baerthlein (8) and Arkwright (2) have mentioned permanence in some cases and not in others. Regarding the reversion of a saltant to its parent type fewer instances are known, and in some of these cases work was not carried out on pure line strains. Reversion has, however, been obtained by Penfold (25), Baerthlein (3), Wreschner (33). The work of Feiler (10) on *B. typhosus*, of Jordan (17) on *B. paratyphosus* B, and of

Soule (30) on *B. subtilis*, is of special interest in this connexion. In the present research, it has been pointed out that there was a partial reversion to the parent type.

SUMMARY.

1. Saltation has occurred in cultures of *B. violaceus* resulting in the formation of a purple and a white sector in the colony.
2. The white strain has again shown saltation to yield two other white sectors distinguished from the parent in the one case by a dry matt surface and in the other by a transparency not possessed by the parent.
3. With the exception of a few minor differences the purple and white strains have been shown to be unchanged from the parent morphologically, physiologically and serologically.
4. Partial reversion on prolonged incubation at air temperature in daylight has occurred in all the saltants when cultivated on potato-extract-glucose-peptone agar.

In conclusion the author desires to express his thanks to Dr. R. St. John Brooks and his assistant, Miss K. M. Rhodes, of the Lister Institute for their kindness in making animal inoculations and preparing the immune sera; also to Professor S. G. Paine who suggested this research, for his constant help and advice, and to Mr. H. Tooley for his able assistance in taking the photographs.

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EXPLANATION OF PLATE XLIII.

Illustrating Dr. J. C. Ramchandani's paper on Saltations in Bacteria. III.

Fig. 1. Petri dish with two colonies of the parent and two colonies of the impure purple saltant showing violet streaks at the margin.

Fig. 2. Petri dish showing colonies arising from mixtures of the violet parent, V_1 , and the white saltant, V_2 ; none of which resembles the purple saltant V_1 .

Fig. 3. Colony of the first saltant white strain with three sectors of matt surface, V_{2a} , and one of more transparent white, V_{2b} .

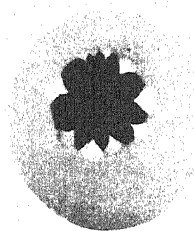
Fig. 4. Gelatine stabs showing the amount of liquefaction caused by the various strains.

Fig. 5. Colony of *Bacillus violaceus* surrounded entirely by sectors, seven white and three purple.

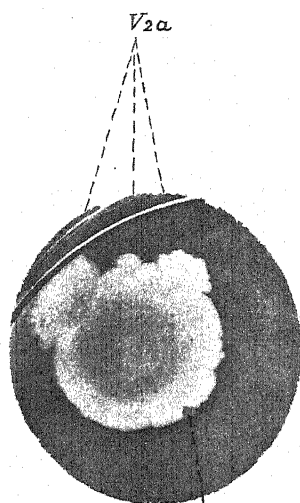
Fig. 6. Colony of the parent showing impure purple and pure white saltant side by side.

Fig. 6 a. Another example of saltation in the parent strain with sectors of pure purple and pure white saltant strains.

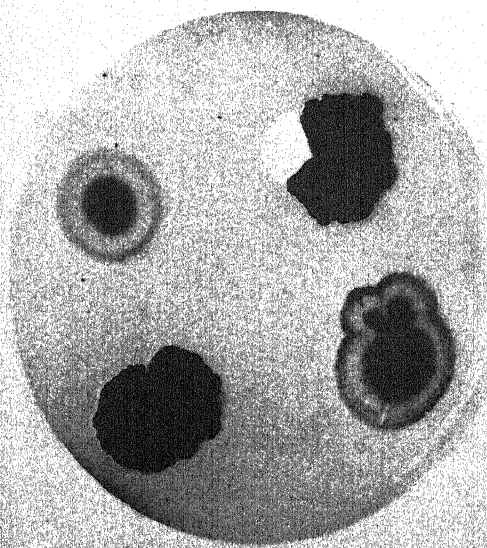
Fig. 7. Compound sector in which two-thirds of the area was purple and one-third was white. The suggestion is given that of the first pair of daughter-cells arising by the division of the saltant, one developed jointly with a violet unaltered cell producing the purple part, while the other developed without admixture with the parent.



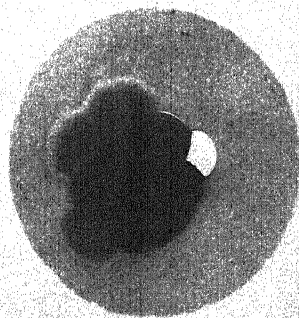
5



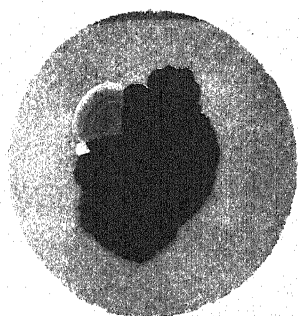
3



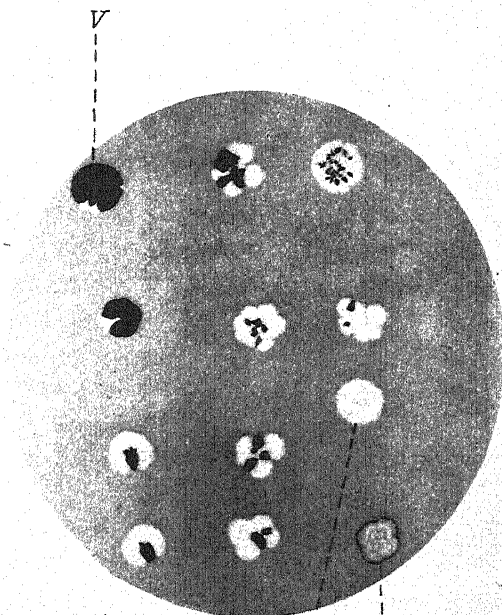
1



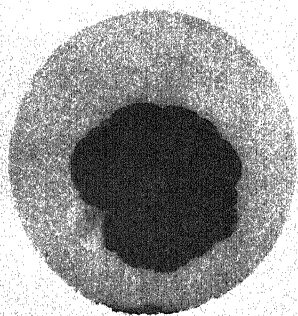
6A



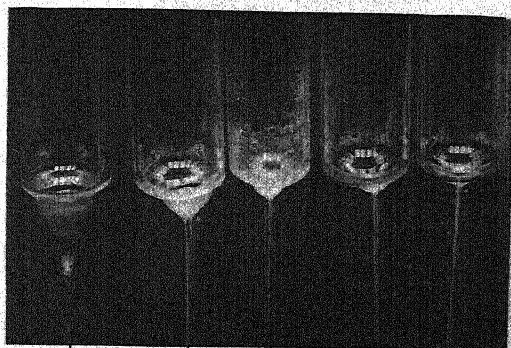
6



2



7



V

V₁

V₂

V_{2a}

V_{2b}

4

The Effect of Ionized Air on the Rate of Respiration of Fungi.

BY

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London.)

With one Figure in the Text.

IN considering the effect of an overhead electric discharge on the growth of plants, such as described by Blackman and his co-workers (1 and 2), the question of the action of ionized air naturally arises, since the high-tension discharge employed in these experiments brings about a considerable ionization of the air. The effect of ionized air on the respiration of plants has been investigated in this laboratory by Middleton (4) in the case of barley seedlings and by Whimster (5) in *pelargonium* leaves.

It seemed desirable that the effect of ionized air on such simple plants as fungi should be investigated; the experiments here described were therefore undertaken. The literature on the subject is well reviewed by Middleton (4).

A. EXPERIMENTS WITH THE PFEFFER-PETTENKOEFER METHOD.

EXPERIMENTAL TECHNIQUE.

The fungi used in these experiments were *Phycomyces Blakesleeanus* and *Polyporus destructor* because the author was well acquainted with the behaviour of these as a result of earlier work (3). The former fungus was cultivated on a thin layer of ground linseed, the latter on flat pieces of carrot in a similar way as in the previous work.

The cultures were used for an experiment when their respirative rate was in the flat part of the curve of the grand period of respiration or just afterwards when the respiration was slowly decreasing.

The method which was employed was the ordinary Pfeffer-Pettenkofer method. CO₂-free air was sucked through the respiration vessel and after leaving this passed through Pettenkofer tubes filled with baryta. Readings were made every hour. The arrangement and routine of the experiments were the same as in the author's previous investigations (3, pp. 118, 209 &c.). The rate of the air current was about 3 litres per hour.

The inner dimensions of the glass respiration vessel were $14 \times 9 \times 4$ cm., its volume being about 500 cc. The top was carefully ground with carborundum so that with the aid of vaseline air-tight closure could be made with a bakolite lid, pressed on it by means of two metal clamps. In the lid were two holes fitted with rubber corks, with the inlet and the outlet tube as is shown in the accompanying figure.

The strength of the baryta was about $\frac{1}{15}$ N. and was titrated against $\frac{1}{40}$ N. HCl, which in its turn was estimated by $\frac{1}{20}$ N. NaOH, standardized with $\frac{1}{20}$ N. oxalic acid.

Ionization. The air was ionized by means of polonium (radium F.) which gives off only α particles which have a range of less than 4 cm. so that at the distance at which it was employed any effect on respiration could result only from the ionized air and not from the α particles directly. Failure to observe any effect on respiration might be due to the fact that the right degree of ionization was not employed. It was therefore determined to investigate various degrees of ionization, starting with a very low degree and gradually working up to very high levels. A very active layer of polonium, deposited on sheets (2.5×2.5 cm.) of copper foil, was employed.

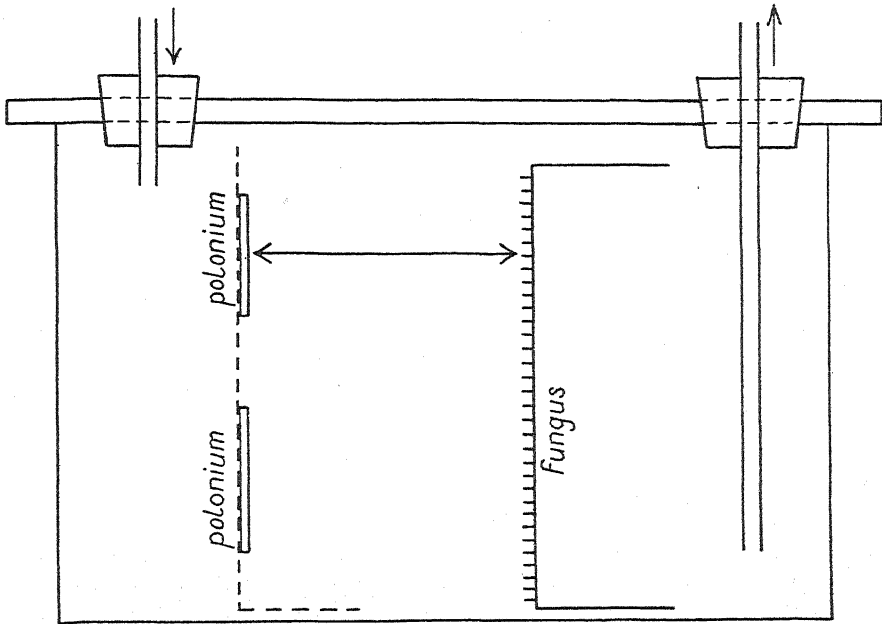
In the preliminary experiments a method of applying the polonium similar to that used by Whimster (5) was employed. The air could be ionized without opening the vessel by lifting a cover from the polonium by means of a rod fixed in a cork in the lid. The layers of culture medium on which the fungus was growing were placed lengthways in the respiration vessel in the same manner as the leaves in Whimster's experiments. This method was, however, abandoned for the following reason.

If an effect of ionized air on respiration occurs it might be expected to vary with the degree of ionization. With the above method it is not possible to determine the optimal degree of ionization because the different parts of the plant are exposed to air of different degrees of ionization. The number of ions in the air decreases very rapidly as the distance from the polonium increases, owing to their rapid recombination. If, for instance, the ionization was 1,000,000 times normal air at 4 cm. distance, it would be reduced to about 4,000 times normal air at a distance of 7 cm.

In the Figure on p. 991, a diagram is given of the way in which the plant was exposed to the polonium. The culture is in a vertical position and parallel to it at a certain distance there is a glass frame to which two pieces of copper foil bearing polonium are attached. Every part of the culture then receives air of approximately the same degree of ionization.

Two pieces of copper foil with polonium were used because the more extended the area of the ionizing agent the larger is the surface of the mycelium parallel to it, and in its turn the larger the readings will be, which makes the results more reliable.

The disadvantage of the arrangement described is, however, that it is not easy to cover or expose the polonium without removing the lid. It was, therefore, simply done by opening the respiration vessel and removing



or replacing the polonium, after which the air was sucked through for half an hour or a little longer and the new series of experiments started.

The respiration vessel has to be taken out of the constant temperature bath in this case and the fungus is exposed to a different temperature for a short time. The whole proceeding, however, does not take more than one to two minutes and the respiration returns to normal within half an hour as was earlier shown with *Phycomyces* (3). Several blank experiments were also carried out with *Polyporus* which showed that the temporary removal of the respiration vessel from the tank and the opening of it, did not affect the respiration, as shown by measurements which were started again half an hour later.

DETERMINATION OF THE DEGREE OF IONIZATION.

The degree of ionization was determined in the usual way. Two copper discs parallel to each other, in this case at a distance of 2 cm., are kept in position by copper wires, well insulated by means of quartz tubing leading through rubber corks. One disc is connected to an electrometer, the capacity of the electrometer and the leads, &c., being 1.3×10^{-10} farad.

The other disc is charged until the so-called saturation current is reached. This current charges the electrometer and from the rate at which the electrometer becomes charged one can calculate the current and so determine the number of ions per cc. The disc connected with the electrometer was surrounded by an earthed guard-ring.

A rotary converter giving a voltage of 1,000 volts and a series of 8 dry batteries giving a voltage of about 100 volts each were available, so that a voltage of about 1,800 volts in all could be obtained.

The measurements of the ionized air gave rise to extreme difficulties. It was expected that a voltage of 100 volts per cm. would suffice to give a saturation current. With the polonium used there is a very high degree of ionization, a saturation current could not be obtained with the highest voltage available. The degree of ionization was, therefore, determined in the following way. Polonium gives off only α rays, which can be stopped by a thick sheet of paper. The polonium was now covered with paper, fixed very closely to it by means of tape, and leaving free areas of 4 mm.², 16 mm.², &c. For the nearer distances such as 4 and 4.5 cm., very accurate measurements could now be obtained, when small areas were used.

It was found that: (1) There is approximately a linear relation between the area of the polonium exposed and the degree of ionization; (2) the degree of ionization decreases according to the distance from the polonium, being reduced to 0.4 of its value for each 0.5 cm. increase, irrespective of the area exposed.

The degree of ionization produced by one piece of polonium at a distance of 4 cm. was found to be about 3×10^6 times normal air under the conditions given and at the particular stage of disintegration of the polonium. Normal air is assumed to contain 500 positive and 500 negative ions per cc.

Any degree of ionization below this one could be obtained and applied to the plants by adjusting the relationship between the distance of the polonium and the area exposed.

The determinations were made in still air, whereas in the actual experiments the air is moving. Both Middleton (4) and Whimster (5) state that no difference was to be observed between measurements in still air and in a current of air; this observation was fully confirmed. An air current, after having passed a tube with CaCl_2 to absorb the water vapour, did not alter the deflections of the electrometer, not even when the air movement was much quicker than in the respiration experiments.

EXPERIMENTAL RESULTS.

All the experiments were carried out at a temperature of 25° C.

The tables showing the results of the experiments are given below and show that the results were negative. The degree of ionization is, of course,

only approximate; especially in the lower degrees, since when two small areas are used, every part of the fungus is not at the same distance from the polonium.

All experiments with *Phycomyces* and the greater part of those with *Polyporus* were performed several times, all with the same negative result.

Several experiments were carried on with the polonium nearer to the fungus than 4 cm. Table XII is an example. The distance was about 2 cm. and if the degree of ionization increased 2.5 times for every $\frac{1}{2}$ cm. the ionization would be about 1×10^8 times normal air. The degree of ionization is, in any case, extremely high, but neither this nor the α rays seem to affect the respiration.

Respiration Experiments with Polyporus destructor.

TABLES I-XII.

The degree of ionization is given in terms of normal air.
(500 pos. + 500 neg. ions per c.c.)

TABLE I.

Ionization = 200-250.

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
2.00-3.00	5.4
3.00-4.00	5.55
4.00-5.00	5.5
<i>Ionization.</i>	
5.45-6.45	5.35
6.45-7.45	5.6
7.45-8.45	5.6
<i>Normal.</i>	
9.30-10.30	5.6
10.30-11.30	5.65

TABLE III.

Ionization = 2000.

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
1.00-2.00	6.15
2.00-3.00	6.0
3.00-4.00	5.7
4.00-5.00	5.9
<i>Ionization.</i>	
5.30-6.30	5.75
6.30-7.30	5.9
7.30-8.30	6.0
<i>Normal.</i>	
9.00-10.00	6.0
10.00-11.00	5.9

TABLE II.

Ionization = 750.

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
11.20-12.20	6.3
12.20-1.20	6.45
1.20-2.20	6.45
2.20-3.20	6.3
<i>Ionization.</i>	
4.00-5.00	6.3
5.00-6.00	6.3
6.00-7.00	6.5
<i>Normal.</i>	
7.30-8.30	6.5

TABLE IV.

Ionization = 5000.

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
12.00-1.00	7.85
1.00-2.00	7.75
2.00-3.00	7.9
<i>Ionization.</i>	
3.30-4.30	7.9
4.30-5.30	7.9
5.30-6.30	8.1
6.30-7.30	8.3

TABLE V.

Ionization = 10,000.

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
12.30-1.30	5.6
1.30-2.30	5.6
2.30-3.30	5.3
3.30-4.30	4.7
<i>Ionization.</i>	
5.10-6.10	4.7
6.10-7.10	4.6
7.10-8.10	4.6
<i>Normal.</i>	
8.45-9.45	4.5
9.45-10.45	4.3

TABLE VII.

Ionization = 75,000.

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
11.00-12.00	8.4
12.00-1.00	8.55
1.00-2.00	8.0
2.00-3.00	8.15
<i>Ionization.</i>	
3.30-4.30	8.2
4.30-5.30	8.5
5.30-6.30	8.15
<i>Normal.</i>	
7.00-8.00	8.25
8.00-9.00	8.1

TABLE IX.

Ionization = 500,000

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
11.45-1.00	5.9
1.00-2.15	5.6
2.15-3.30	5.8
3.30-4.45	5.8
<i>Ionization.</i>	
5.30-6.45	5.6
6.45-8.00	5.7
8.00-9.15	5.6
9.15-10.30	5.7

TABLE VI.

Ionization = 25,000.

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
12.00-1.00	4.4
1.00-2.00	4.1
2.00-3.00	4.1
<i>Ionization.</i>	
3.30-4.30	4.1
4.30-5.30	4.1
5.30-6.30	3.8
<i>Normal.</i>	
7.00-8.00	3.8
8.00-9.00	5.65

TABLE VIII.

Ionization = 150,000.

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
12.00-1.30	5.8
1.30-3.00	5.6
3.00-4.30	5.6
4.30-6.00	5.7
<i>Ionization.</i>	
6.30-8.00	5.7
8.00-9.30	5.7
9.30-11.00	5.8

TABLE X.

Ionization = circa 1,250,000

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
11.00-12.30	6.7
12.30-2.00	6.8
2.00-3.30	6.8
3.30-5.00	6.7
<i>Ionization.</i>	
5.45-7.15	6.7
7.15-8.45	6.7
8.45-10.15	6.5

TABLE XI.

Ionization = 3-4,000,000.

Hour.	CO ₂ (c.c.)
<i>Normal.</i>	
12.15-1.15	7.3
1.15-2.15	7.1
2.15-3.15	6.8
3.15-4.15	6.8
<i>Ionization.</i>	
5.00-6.00	6.7
6.00-7.00	6.6
7.00-8.00	6.7
8.00-9.00	6.6

TABLE XII.

Ionization = 100,000,000 (?).

Hour.	CO ₂ (c.c.)
<i>Normal.</i>	
10.45-12.00	7.0
12.00-1.15	7.1
1.15-2.30	7.2
2.30-3.45	7.3
<i>Ionization.</i>	
4.30-5.45	7.2
5.45-7.00	7.1
7.00-8.15	7.2
8.15-9.30	7.2

TABLES XIII-XXIV.

Experiments with Phycomyces Blakesleeanus.

The degree of ionization is given in terms of normal air.

TABLE XIII.

Ionization = 500.

Hour.	CO ₂ (c.c.)
<i>Normal.</i>	
11.00-12.00	5.5
12.00-1.00	5.7
1.00-2.00	5.7
2.00-3.00	5.6
<i>Ionization.</i>	
3.30-4.30	5.5
4.30-5.30	5.7
5.30-6.30	5.6
<i>Normal.</i>	
7.00-8.00	5.6
8.00-9.00	5.7

TABLE XIV.

Ionization = 1,000.

Hour.	CO ₂ (c.c.)
<i>Normal.</i>	
11.00-12.00	6.1
12.00-1.00	5.75
1.00-2.00	5.6
2.00-3.00	5.6
<i>Ionization.</i>	
3.30-4.30	5.35
4.30-5.30	5.35
5.30-6.30	5.35
<i>Normal.</i>	
7.00-8.00	4.95
8.00-9.00	4.8

TABLE XV.

Ionization = 2,000.

Hour.	CO ₂ (c.c.)
<i>Normal.</i>	
11.25-12.25	7.8
12.25-1.25	7.45
1.25-2.25	7.25
2.25-3.25	7.45
<i>Ionization.</i>	
4.00-5.00	7.4
5.00-6.00	7.2
6.00-7.00	6.65
<i>Normal.</i>	
7.35-8.35	6.4
8.35-9.35	6.1

TABLE XVI.

Ionization = 4,000.

Hour.	CO ₂ (c.c.)
<i>Normal.</i>	
12.00-1.00	8.25
1.00-2.00	8.35
2.00-3.00	8.35
3.00-4.00	8.35
<i>Ionization.</i>	
4.35-5.35	8.35
5.35-6.35	8.85
6.35-7.35	9.2
<i>Normal.</i>	
8.10-9.10	9.2
9.10-10.10	9.6

TABLE XVII.

Ionization = 8,000.

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
11.25-12.25	7.95
12.25-1.25	7.95
1.25-2.25	7.85
2.25-3.25	7.95
<i>Ionization.</i>	
4.00-5.00	7.85
5.00-6.00	7.8
6.00-7.00	7.8
<i>Normal.</i>	
7.30-8.30	7.7
8.30-9.30	7.85

TABLE XVIII.

Ionization = 16,000.

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
12.30-1.30	9.25
1.30-2.30	9.1
2.30-3.30	8.8
<i>Ionization.</i>	
4.10-5.10	8.4
5.10-6.10	8.0
6.10-7.10	7.45
<i>Normal.</i>	
7.45-8.45	7.0
8.45-9.45	6.5

TABLE XIX.

Ionization = 30,000.

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
12.00-1.00	9.0
1.00-2.00	8.7
2.00-3.00	8.7
3.00-4.00	8.55
<i>Ionization.</i>	
4.30-5.30	8.4
5.30-6.30	8.4
6.30-7.30	8.2
<i>Normal.</i>	
8.00-9.00	8.0
9.00-10.00	7.85

TABLE XX.

Ionization = 100,000.

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
10.30-11.30	6.4
11.30-12.30	6.6
12.30-1.30	6.0
1.30-2.30	6.1
<i>Ionization.</i>	
3.00-4.00	6.0
4.00-5.00	6.3
5.00-6.00	6.2
<i>Normal.</i>	
6.30-7.30	6.6
7.30-8.30	6.2

TABLE XXI.

Ionization = 200,000.

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
10.00-11.00	9.6
11.00-12.00	9.6
12.00-1.00	9.6
1.00-2.00	9.7
<i>Ionization.</i>	
2.30-3.30	9.8
3.30-4.30	9.8
4.30-5.30	9.8
5.30-6.30	9.6
6.30-7.30	9.6

TABLE XXII.

Ionization = 500,000.

Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
10.30-11.30	7.2
11.30-12.30	7.2
12.30-1.30	7.2
1.30-2.30	7.5
<i>Ionization.</i>	
3.10-4.10	7.2
4.10-5.10	7.5
5.10-6.10	7.5
6.10-7.10	7.2

TABLE XXIII.

Ionization = 1,500,000.	
Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
11.45-12.45	7.8
12.45-1.45	7.6
1.45-2.45	7.2
2.45-3.45	7.2
<i>Ionization.</i>	
4.30-5.30	6.9
5.30-6.30	6.8
6.30-7.30	6.7
<i>Normal.</i>	
8.10-9.10	6.3
9.10-10.10	6.2

TABLE XXIV.

Ionization = 3,000,000.	
Hour.	CO ₂ (c.c.).
<i>Normal.</i>	
12.00-1.00	6.0
1.00-2.00	6.1
2.00-3.00	5.9
<i>Ionization.</i>	
3.45-4.45	5.9
4.45-5.45	6.1
4.45-6.45	6.0
<i>Normal.</i>	
7.15-8.15	5.8
8.15-9.15	5.9

DISCUSSION OF RESULTS.

There is not the slightest sign of an effect of ionized air on the respiration. In some experiments the respiration decreases but this is due to the fact that the optimum of the grand period has been passed. The readings then become gradually smaller and there are no changes after the application or removal of the polonium.

There are no experiments at a degree of ionization below 200 times normal air since such could not be measured very accurately. But some experiments were carried out with the fungus still further away from the polonium than the distance required to give an ionization of 200, so that the ionization must certainly have been smaller; there was no effect, however.

The determinations of the degrees of ionization were all made in very dry air because of the great leakage of current in damp air. The fungi are, however, exposed to a very damp atmosphere during the respiration experiments. It may be that in damp air the ions recombine more quickly than in dry air, so that the cultures were not exposed to the degree of ionization assumed in the tables. There would, however, in such an experiment as that of Table XII be a sufficient degree of ionization, to show an effect if any occurred.

Some experiments were carried out with CaCl₂ on the bottom of the respiration vessel and with dry air entering. The culture dried up gradually and the respiration decreased rapidly after some hours, following a smooth falling curve. Polonium applied at a certain time did not alter in any way the regular shape of the curve.

B. EXPERIMENTS WITH THE MICRO-RESPIROMETER.

The experiments described under section A give no information concerning respiration during the first half hour of exposure to ionized air.

Experiments with a so-called micro-respirometer were, therefore, carried out to observe the effect and power of ionized air during the early period of exposure.

The micro-respirometer employed consisted of the usual experimental and compensation vessels. The capillary, in which a small drop of paraffin oil, coloured with Sudan III, moves, was provided with a scale in which a movement of 1 mm. corresponds to a change in volume of 0.0006 cc. at 25° C.

The bottom of the experimental vessel is filled with a block of sealing-wax, supporting a small glass dish containing strong sodium hydroxide solution; the same solution is in the compensation vessel. A narrow glass tube, closed at the bottom, is embedded vertically in the block of sealing-wax, and contains ionium, the amount of which can be varied. The end of this tube projects slightly from the surface of the sealing-wax and is covered by a small glass cup which fits in a groove in the wax round the end of the tube.

During the experiments this cup can be removed, without opening the experimental vessel, by the use of a strong magnet attracting a thin iron sheet attached to the cup. This does not take longer than a few seconds.

A small layer of ground linseed with *Phycomyces* is placed on a small glass stand in the experimental vessel with the mycelium facing downwards.

The amount of ionized air was measured in a small glass bottle of the same size as the experimental vessel, with the same sealing-wax block and two copper discs of 1 sq. cm. in the position occupied by the fungus during the experiments.

The glass cup appeared to close well, for if there had been a leak giving as small a degree of ionization as 50 times normal air this would have been detected by the electrometer.

The following tables give the results of some experiments.

TABLES XXV-XXVII.

Experiments with the Micro-respirometer.

Respiration of *Phycomyces Blakesleeanus* at 25° C.

Readings on scale of micro-respirometer at intervals of *three minutes*.

Each unit equals 0.1 mm.

The figures in italics are for the periods of ionization.

TABLE XXV.

Ionization = 1,400.

Scale Readings: 98, 99, 98, 99, 99, 101, 100, 100, *101, 100, 99, 99, 98.*

TABLE XXVI.

Ionization = 8,000.

Scale Readings: 98, 98, 100, 99, 98, 97, 98, 99, 97, 98, 99, 99, 100, 101, 100, 98, 99, 98, 98, 98.

TABLE XXVII.

Ionization = 20,000.

Scale Readings: 118, 121, 119, 120, 121, 122, 121, 119, 121, 121, 120, 122, 121, 123, 122, 121.

It will be seen that the rate of movement of the column of liquid in the micro-respirometer is not affected by exposure of the fungus to ionized air, i.e. no effect on respiration is to be observed.

SUMMARY.

The effect of ionized air on the rate of respiration of *Phycomyces Blakesleeanus* and *Polyporus destructor* was studied, the respiration being determined both by the method of absorption by baryta and by a micro-respirometer. The degree of ionization employed varied from a few hundred to several million times that of normal air.

No effect on the rate of respiration was to be observed in any of the experiments.

The writer wishes to record his thanks to Professor V. H. Blackman for much help and interest in the progress of the work.

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The Resistance to Poisons of Desiccated Plant Tissues.

BY

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With Plate XLIV.

THE experiments here described arose from an observation made by Dr. E. J. Schwartz, that a piece of moss after a sojourn in absolute alcohol was found growing in his garden some weeks after it had been thrown away. He suggested that the matter was worth further investigation.

It has been known for some considerable time, that, provided certain plant structures are carefully desiccated, they can withstand treatment at high temperatures which would prove fatal under normal conditions.

It is well known that mosses—with few exceptions—can withstand intense drought, or desiccation, for considerable periods, and yet recover on being provided with water.

In all the experiments quoted below, the moss used was very carefully dried. Towards the end of the drying process it was subjected to gentle oven temperatures. The moss, when thoroughly desiccated, was in a very brittle condition.

The results here given, it should be understood, are only a small proportion of those obtained. Throughout the experiments described here the material used was *Mnium hornum*.

Series A.

The material here used was carefully air-dried as stated above, and then divided into a number of portions. Only three such sections need be given in full, and these for convenience we will call A 1, A 2, and A 3. A 1 was treated with absolute alcohol for one hour, A 2 for two hours, and A 3 for thirteen hours.

The material was placed in small glass tubes with well-fitting corks, and covered carefully with the alcohol. Several changes of alcohol were made. At the end of the time allowed, the specimens were plunged directly into tap water, and thoroughly washed. No effort was made to

dilute the alcohol gradually ; on the contrary, the transition from alcohol to water was made as suddenly as possible. The material was then divided up into a number of portions of a convenient size, and each portion was planted in a pot about half full of soil.

Experience showed that, in order to obtain good results, the pot should be covered with a sheet of glass, so that the atmosphere surrounding the specimen was saturated. The damp chamber required was best obtained by planting the specimen so that it reached within about half an inch of the glass placed on top of the pot. Each pot was watered from time to time, but it was found best to keep the soil below saturation. Watering was therefore carried out about twice each week. All the pots were set aside in a shady place. The results are presented in the following table.

TABLE I.

Interval from planting.	A 1.	A 2.	A 3.
8 weeks	One very small shoot.	No sign of life.	No sign of life.
10 "	"	" "	" "
12 "	Shoot slightly longer.	" "	Three very " small shoots.
13 "	Shoot still growing.	One minute shoot.	Four " " "
14 "	A further shoot appeared.	Shoot still small.	Specimen nearly rotten, but the four shoots now slightly longer.
15 "	Four shoots in all.	" "	Do. but five shoots now present.
20 "	Numerous small sturdy shoots.	One long shoot and six small shoots.	Most of specimen rotten but shoots still persist.

A plant of a control experiment which was set up, put forth several shoots at an early stage, and had produced a mass of shoots at the end of eight weeks.

The above brings out clearly the fact that, while the material treated with alcohol suffered, yet it was not killed after a period of thirteen hours in alcohol.

Later, when the above series was repeated, a quantity of material was subjected to the action of absolute alcohol for eighteen hours. Even in this case the moss was able to put forth several shoots, but only after an interval of about fourteen weeks.

Series B.

In this series the effect of dilute alcohol was tested. A quantity of material, having been previously dried, was treated with 50 per cent. for one hour. The material was then washed and planted as before. In all, only one green shoot appeared in all the material so treated—and that after an interval of thirteen weeks. The rest rotted away.

A very similar result was obtained when the concentration of alcohol was increased to 90 per cent. Here, again, the material only produced a single shoot—which, however, was half an inch long after an interval of fifteen weeks.

The results of the above series of experiments have been borne out time and time again. It would seem that the presence of any water is sufficient to cause the alcohol to kill the material, although absolute alcohol for eighteen hours failed to do this.

Series C.

Since the moss could evidently withstand alcohol, it was decided to try the effect of xylol. In this case after the usual thorough air-drying, the material was placed in absolute alcohol for one hour. The material was then divided into two portions, C 1 and C 2. In the case of C 1 the material was gradually taken up into xylol, until a maximum concentration of 50 per cent. was reached. At the end of half an hour, alcohol was added, and the concentration of xylol thus gradually reduced. In all, the material was in xylol of various concentrations for about one and a half hours. Having been cleared of xylol, it was washed in water.

In the case of C 2, the transition from alcohol to xylol and back again was made as suddenly as possible. The material was placed in absolute alcohol for one hour, and then plunged direct into pure xylol. Several changes of xylol were given, and the total time allowed in the xylol was two hours. A thorough washing in water was given, following that in alcohol.

The records below were taken from pots which were considered to give a fairly average result. The control experiments set up were of two varieties—some had been simply dried, and then planted, while others had undergone an hour in alcohol. These controls behaved in every way as was anticipated, and can therefore be dismissed.

TABLE II.

Interval after planting.	C 1.	C 2.
8 weeks	Four very small shoots.	Five short green shoots.
10 "	Shoots only slightly longer..	Six "
12 "	" " " " " "	Some elongation to be observed.
13 "	Shoots elongated, but practically devoid of leaves, except near their apices.	Further shoots appeared. Some of the earlier shoots now long and weak.
14 "	Shoots still long and thin.	Several shoots died away. (Three left.)
15 "	Now five shoots in all.	Further shoots appeared.
19 "	Six shoots—five about $\frac{1}{2}$ inch long and one just showing.	Two of the five shoots very long (1 inch) and slender.

It was unfortunate that in the C 2 series chosen, some of the shoots should have died away. This, however, was not an unusual occurrence. It can only be attributed to the fact that conditions of temperature and humidity which might favour the appearance of the shoots, did not always encourage their continued growth. The very slender nature of all the shoots which arose—even in the controls—clearly showed that the moss was living under somewhat 'hot-house conditions'.

The above results, however, tend to show that even xylol cannot harm the plant while the latter is in the dormant condition induced by extreme desiccation.

Series D.

The effect of chloroform was now tried. The material, having been air-dried, was placed in absolute alcohol for one hour, and then divided as follows:

D 1. A few drops of chloroform only were added to the alcohol, and at the end of fifteen minutes, the material was thoroughly washed in water.

D 2. The material was suddenly placed in strong chloroform, by emptying away the alcohol, and pouring on chloroform. In this case the concentration of chloroform was probably in the region of 90 per cent. The time allowed in chloroform was again fifteen minutes. The material was then plunged into water and thoroughly rinsed.

D 3. In this case the material was placed in absolute chloroform for one hour. Several changes of chloroform were given during that period, so that the concentration was probably in the region of 100 per cent. As soon as the treatment in chloroform was completed, the moss was plunged into water.

It should be noted that chloroform is only soluble in water to the extent of about 1 per cent. An attempt was therefore made, by washing very rapidly in water to remove all trace of the chloroform before the dormant condition of the protoplasm was affected by the water.

D 1. All the material treated as described above under this heading was successful, and produced a large number of shoots, although some of these were very weak and slender.

D 2. After a lapse of about eight weeks, a very few minute shoots were visible, but these all gradually died away.

D 3. The whole of this series only produced one small shoot. It was most unfortunate that the material was not washed in alcohol after treatment with chloroform, and before being washed in water. Experiments are, as a matter of fact, being conducted on those lines, and results already obtained are more satisfactory.

It would appear that a change takes place directly the water pene-

trates the tissues, and that in this condition the protoplasm is readily injured by chloroform. The 'latent period' as it might be termed, between immersion in water, and resumption of protoplasmic sensitivity must be very brief indeed. It is apparently long enough to permit of alcohol being washed away, but not long enough to allow chloroform to be removed. It seems improbable that it is a question of the relative powers of alcohol and chloroform to injure the plant under normal conditions. This view is borne out by the fact that, in several cases where the alcohol was washed away slowly, the results were of the poorest. It was found necessary to wash very rapidly indeed, and with a complete disregard of the possible action of surface tension on the tissue. Were chloroform as easily miscible with water, as is alcohol, I do not doubt but that the last series of experiments given would have met with a better result.

Series E.—Alcohol and Acetone.

Here the material was air-dried, and placed in alcohol for one hour. It was then divided into five distinct portions, and treated as follows:

E 1. A few drops of acetone were added to the moss while in alcohol, and the material kept under these conditions for half an hour. The acetone was then washed out with alcohol and, finally, the material plunged into water.

E 2. Material treated exactly as in *E 1*, except that in this case, the moss was plunged into water direct from the acetone solution.

E 3. Here the moss was allowed an immersion of thirty minutes duration in absolute acetone. It was then transferred back to alcohol, and finally washed.

E 4. Material treated as in *E 3*, but as in the case of *E 2*, no intermediate stage in alcohol was given between acetone and water.

E 5. After the initial treatment with alcohol, the material was placed in absolute acetone for one hour. It was then plunged directly into water. Again, in the above, average material was chosen for purposes of result.

E 1. As might be expected this series developed well. Many healthy shoots were put forth, and all continued to progress. This particular series, it might be remarked, was more successful as a whole, than any of the series treated with alcohol alone.

E 2. This series though quite successful, did not produce such healthy shoots as the previous series. The obvious interpretation which one might be tempted to place on this fact, however, scarcely seems to hold good when the results of *E 3* and *E 4* are compared.

E 3. Here also, a fair measure of success was attained, although the shoots were not so robust as those in *E 1*.

E 4. The result here was very marked; the plants very nearly attained the standards reached by those of *E 1*.

E5. The plants here progressed as well as those in E4.

It would seem to be fairly evident that acetone (at any rate when allowed to act for the period stated) has little toxic effect on the moss.

Series F.—Alcohol and Ether.

The material used was thoroughly air-dried, and treated with absolute alcohol for one hour.

The alcohol having been poured off, ether was added, and several changes given. The material was kept two hours in the ether as a maximum, and it will be sufficient for our purpose if we consider only the specimens so treated. After immersion in ether, the material was thoroughly washed in alcohol and, finally, plunged into water.

The result may be very briefly stated. All the material treated with ether gave a positive result, that which had been immersed for two hours being practically as successful as that which had only undergone half an hour's treatment.

General Considerations.

It will be noted that, in all the above experiments, alcohol was used. It should be understood that, once the fact had been fully established that alcohol itself—at any rate for moderate periods—was practically harmless to the dried protoplasm, it was simply used as an additional dehydrating agent in all the further experiments quoted. As stated above, some material immersed in alcohol for eighteen hours, recovered in a very satisfactory manner. The results for periods greater than eighteen hours are rather doubtful, although such have been obtained. For the present eighteen hours will be taken as a maximum.

Objection may be made that (*a*) the alcohol and other materials did not penetrate the tissues, (*b*) spores, and not the tissues themselves, survived the treatment.

Objections under (*a*) might be further subdivided as follows: that the alcohol had not time to penetrate the tissues, and that air bubbles caught up in the tomentum might have protected the tissues.

As to whether the time allowed was sufficient it may be pointed out that positive results have been obtained after an immersion of eighteen hours in absolute alcohol. One may assume that complete penetration had taken place during that period. In any case, it is extremely doubtful if the more central cells of the stem give rise to the new growths, as will be mentioned later. It seems rather, that it is the cells towards the circumference of the stem and, perhaps, in some cases, the cells of the ramentum that have produced these growths. It may safely be taken that these latter cells were penetrated by the fluids.

Regarding the question of the possible presence of air-bubbles in the tomentum, it may be stated that everything possible was done to guard against this. After the original air-drying, the stems were pulled apart, and much of the tomentum removed. In every case also, the tubes in which the specimens were placed were completely filled with the liquid concerned, and a good-fitting cork was used. The tubes were placed at different angles (e.g. upside down, &c.) from time to time, to prevent any air-bubbles adhering, and as far as one could tell no air-bubbles whatever were present. Further, if air-bubbles protected the material in absolute alcohol, one would have expected protection in the same way, in the case of dilute alcohol which proved fatal.

It may be here noted that the tomentum was not so carefully removed from the material set aside for controls, and this probably accounted for the greater number of shoots put forth by the controls, as opposed to the others.

Objections under (*b*) that it was the spores and not the tissues themselves which survived, have now to be considered. In many cases a shoot would make its appearance after the majority had shown themselves, often in a totally different part of the specimen, and with no suggestion of a protonemal connexion between the two areas. Were the distant shoot simply a bud formed from a protonema produced by a spore, the connexions would be easy to follow, since the general hue of the specimens is a dark brown, while that of any new part is a dark green.

One would further expect all spores which withstood the treatment to germinate within a very limited period of each other, and the inference is, that if the main body of shoots were due to the survival of spores, then the appearance of later shoots could not be due to the same cause. As a matter of fact, I found no evidence to support the view that any of the shoots were produced from spores. The shoots on all controls arose in exactly the same way as did those in the others, and it is allowed that the moss itself—and not only its spores—will withstand desiccation.

As an additional, and fairly reliable precaution against the presence of spores, material used was gathered and allowed to remain under ordinary conditions of growth for about a month before drying was begun. It was considered that any spores present should have germinated in that time. Further, no material was gathered which bore old capsules.

It was decided at an early stage in these investigations that, as an additional precaution, sterilized soil should be used. Further, the tap-water used to water the specimens was filtered. The glass covers placed over the pots were only removed for brief periods of watering and inspection. It will therefore, probably be agreed, that the chances of spores being responsible for the growths produced, are very remote.

The new growths produced may be described in greater detail, and it

would seem that the method of branching in *Mnium hornum* is worthy of further investigation. While it is most difficult to be certain in this matter, it would appear that certain cells on the periphery of the stem produced a short tomental outgrowth or outgrowths, and that these latter very early produced a bud, giving rise of course, to the shoot. To the naked eye these shoots usually appeared to arise directly from the stem, and this probably was actually true in a few cases. But in the majority of cases, the slightest force was sufficient to detach the shoot from the parent stem. Such a detached shoot, under the microscope, always showed the presence at its base of several tomental filaments, which gave the impression of roots at the base of the stem of an ordinary plant. One fact, however, is certain—namely that the mode of origin of the shoots of the controls, and of the experimental material was identical. It is to be regretted that the new shoots arose in such a curious manner, but there is no doubt that it was due to the conditions under which the specimens were grown. In this connexion it is interesting to note that in several cases a bud was found to have grown on a leaf of the old moss—an impossible condition in this type under normal conditions. Such a growth does, however, serve to support the view that the liquids used had actually penetrated the tissues which later revived, for the leaf, of course, is extremely thin.

Another moss has been subjected to treatment along similar lines, and in this case, actual and very definite organic connexion between the shoot and the parent stem has been obtained. It is hoped to present the results of this work later.

The terms which I have used to describe the shoots are purely relative, and without doubt, would not hold good under different conditions of environment of the material during the period of growth.

Experiments are being carried out with a view to determining whether the results obtained among mosses, are applicable to other plants and parts of plants (e. g. seeds) which can normally withstand desiccation.

Finally, I should like to express my very sincere thanks to Dr. E. J. Schwartz, who has afforded me the most valuable assistance. I am further deeply indebted to Prof. Dame Helen Gwynne-Vaughan, and to the late St. John Marriott for their helpful criticism.

CONCLUSIONS.

Mnium hornum, when thoroughly desiccated, can withstand the action of certain substances which would prove lethal under normal conditions, i. e. when water is present.

The desiccated moss was able to grow after eighteen hours' immersion in *absolute* alcohol; dilute alcohol was, however, fatal.

